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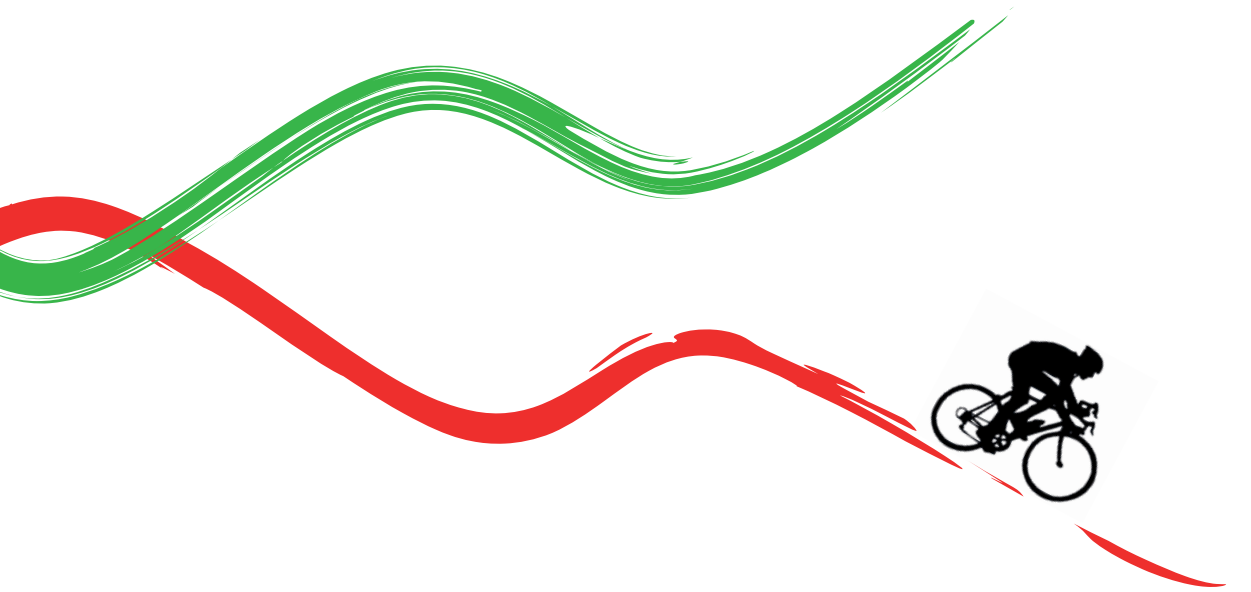

On the physiological and psychological differences between functional overreaching and acute fatigue

Twan P. G. ten Haaf

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On the physiological and psychological differences between functional overreaching and acute fatigue

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copromotor: dr. J.J. de Koning

"The most important is

to discover

what is the most important"

- Shunryu Suzuki -

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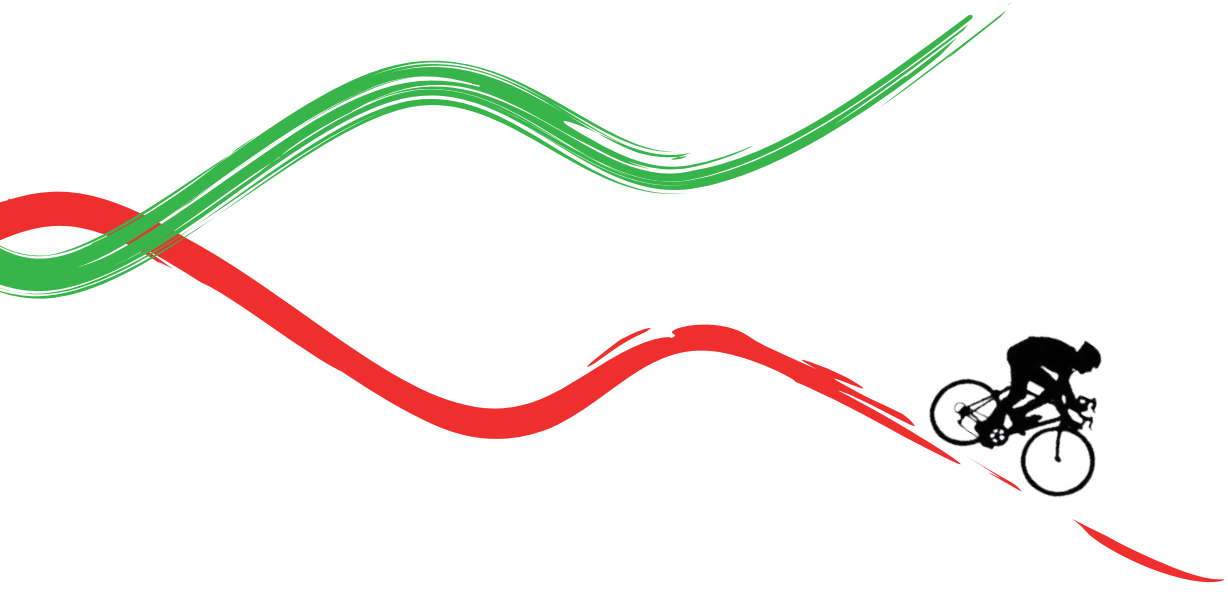
Chapter 1

General introduction



1.1 An anecdote about scientific observations

The history of science is ornamented by some great scientific discoveries made after observations in an ecological setting. Charles Darwin made his famous observations on various species of finches at the Galápagos Islands, which led him to formulate his theory on natural selection and evolution (Darwin 1859). A few years later, the English physician John Snow noticed the geographical distribution of infections during the cholera epidemic in London in 1854. His investigations and intervention (disabling the handle of the infected well pump) elegantly demonstrated the source of the epidemic (Snow 1855).

Similarly, an observation in ecological settings was the starting point of this PhD thesis. Obviously I do not want to draw comparisons between myself or this PhD thesis and the previously mentioned scientific classics, other than that the research arose from observations in an ecological setting. Whereas Snow noted the geographical spread of infections, and Darwin saw the various species of finches, dr. De Koning noticed physiological and psychological changes during his endeavour to cycle the Tour for Life (TFL) in 2013. This fundraising cycling tour from Italy to the Netherlands encompassed a ruthless 1264 km in 8 consecutive days. After a few days of cycling, dr. De Koning noticed that he was unable to perform at his heart rate-based exercise intensity, estimated before the event by means of a maximal incremental exercise test. Back home he observed a reduced ability to perform, persistent fatigue, and an altered mood state. From a literature search, it soon turned out he entered the domain of *overtraining*.

1.2 Effective training or overtraining?

Effective training requires a balance between (exercise-induced) stress and recovery, and results in increased performance capacity. Athletes sometimes increase their training load, for example during training camps. If the increased training load is balanced with sufficient recovery, a period of intensified training

results in acute fatigue (AF). This balanced intensified training is followed by positive adaptation of the affected physiological systems (supercompensation) and increased performance (Figure 1.1). In contrast, increased training load that is not met by adequate recovery results in (temporal) underperformance. Besides increased training load, other factors such as psychosocial stressors or a busy travel and competition scheme may add to the stress-recovery imbalance (Figure 1.1). This process is termed overtraining (Meeusen et al. 2013). Thus, intensified training results either in acute fatigue or overtraining, depending on the balance between (exercise-induced) stress and recovery. It is important to note that the internal training load (i.e. the physiological and psychological stress) that results from a given external training load (e.g. distance or power output) may differ between individuals and between settings (Halson 2014).

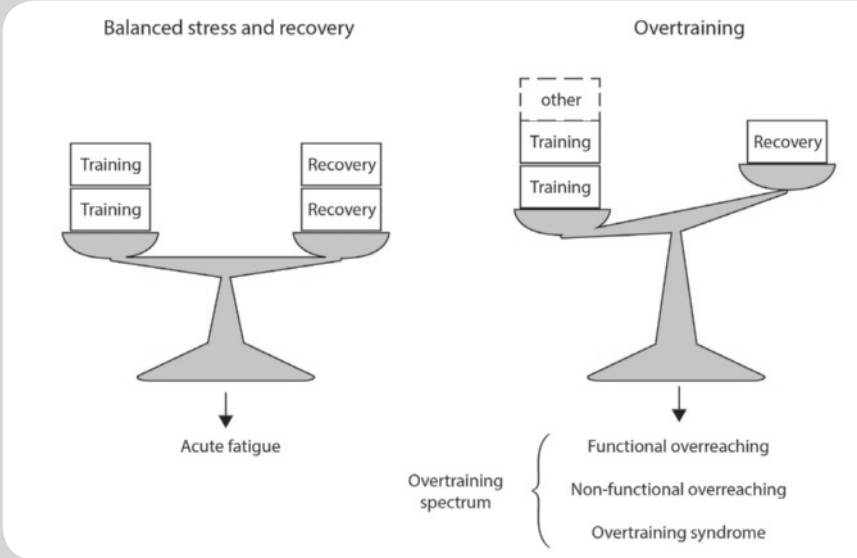


Figure 1.1. Intensified training. Increased training load that is met by appropriate recovery results in acute fatigue and increased fitness. Insufficient recovery results in overtraining, with the possible outcomes functional overreaching (FOR), non-functional overreaching (NFOR), or overtraining syndrome (OTS).

The possible outcomes of overtraining are functional overreaching (FOR), non-functional overreaching (NFOR) or overtraining syndrome (OTS). Underperformance is one of the main characteristics of the outcomes of overtraining, accompanied by several other possible symptoms such as disturbed mood, sleep and immune function (Meeusen et al. 2006; Meeusen et al. 2013). It is unclear whether FOR, NFOR and OTS share (all) symptoms, and whether the symptoms are equally severe in the different stages. Thus, it is not known whether FOR, NFOR and OTS are part of a continuum, or are distinct phenomena (Meeusen et al. 2013). For that reason, in this thesis the terminology 'overtraining spectrum', rather than 'overtraining continuum' will be used. The stages of the overtraining spectrum are retrospectively diagnosed based on the duration of recovery. FOR takes days to weeks to recover, whereas NFOR takes weeks to months, and OTS months to more than a year to recover.

One of the challenges of research on the training-overtraining spectrum is to distinguish between AF and FOR. Some important differences exist between these two stages of the overtraining spectrum. Firstly, intensified training which results in AF leads to increased performance. In FOR athletes, however, the increase in performance after intensified training and a taper period is smaller or even absent (Aubry et al. 2014). Secondly, disturbed sleep and an increased illness incidence in FOR compared to AF athletes has been shown (Hausswirth et al. 2014). Thirdly, continuation of imbalanced training-recovery in FOR athletes results eventually in NFOR or OTS. These stages must be prevented because of the severe symptoms and long recovery times (Meeusen et al. 2013). In conclusion, it is the difference between AF and FOR that is very relevant for athletes, because it represents the thin border between effective and too much training. The aim of this thesis was, therefore, to identify parameters that help to distinguish between AF and FOR athletes. Despite its importance, little is known about the methods to distinguish between AF and FOR. Different factors contribute to this knowledge-gap. Firstly, inconsistent terminology and criteria have been used to describe

overtraining and the possible outcomes FOR, NFOR and OTS. Overtraining and its outcomes have been described as staleness, overtraining, overtraining syndrome, training stress syndrome, or underperformance syndrome (Nederhof et al. 2006). Also, many different criteria have been used to define FOR. Researchers relied on (different combinations of) decreased performance, ratings of fatigue, mood disturbances, increased or decreased heart rate, or decreased maximal blood lactate (Brink, Visscher, Coutts, et al. 2012; Dupuy et al. 2014; Nederhof et al. 2007; Schmikli et al. 2012). A second factor contributing to the knowledge-gap is the limited available data. This is exemplified by a review on hormonal disturbances in overtraining, that included fewer original investigations than the number of reviews found on the topic (Cadegiani and Kater 2017b). Also, the number of subjects included in studies is generally small. This is illustrated by the same review on hormonal disturbances, that reported a median number of subjects in the included studies of 14 (range 3-36). The scarcity of data may be explained by the disturbance overtraining has on an athlete's training schedule. Also, research on overtraining faces ethical issues due to the lack of treatment (except rest) and the severity of symptoms. So, inconsistent terminology and criteria, and the limited available data give rise to the challenge to identify parameters that relate to underperformance after intensified training (FOR).

1.3 The most promising parameters

Many hypotheses on the underlying mechanisms and potential markers of overtraining have been postulated (Armstrong and VanHeest 2002; Gastmann and Lehmann 1998; Lehmann et al. 1998; Petibois et al. 2002; Robson 2003; Smith 2000; Smith 2003; Snyder 1998). Some of these are solely based on theoretical concepts, while others are grounded with data. The most recent consensus statement on overtraining by the European College of Sport Sciences and the American College of Sports Medicine suggested that hormones, heart rate, reaction time and monitoring of stressors and symptoms of overtraining

are amongst the most promising tools to identify FOR (Meeusen et al. 2013). Therefore, the main research question in this thesis was: Can hormonal levels, heart rate, reaction time or monitoring of stressors and symptoms be used to distinguish between acute fatigue and functional overreaching?

Hormonal system

Exercise induces a (neuro)endocrine stress response that facilitates physical performance by affecting metabolism and the cardiovascular system. For example, metabolic fuels are mobilised, and heart rate and pulmonary ventilation increase upon exercise initiation. The two central components in the neuroendocrine and endocrine stress response are the locus coeruleus noradrenergic (LC/NE) sympathetic system and the hypothalamus (Figure 1.2) (Chrousos 1998; Tsigos and Chrousos 2002). Efferent sympathetic neurons from the LC/NE sympathetic system innervate the adrenal medulla, which secretes catecholamines into the circulation upon stimulation (Tsigos and Chrousos 2002). Metanephrine (MN) and normetanephrine (NMN) are metabolites of adrenal epinephrine and norepinephrine, respectively (Eisenhofer et al. 1995). The hypothalamus affects the anterior pituitary by means of several excitatory and inhibitory factors (Elenkov et al. 2000). For example, growth hormone releasing hormone (GHRH) stimulates, whereas somatostatin inhibits somatotrophic cells in the pituitary to release growth hormone (GH). Prolactin (PRL) is secreted from lactotrophic cells that are, amongst other factors, under inhibiting control of dopamine. Corticotropin releasing hormone (CRH) stimulates corticotrophic cells to secrete adrenocorticotropic hormone (ACTH), which in turn stimulates the release of cortisol from the adrenal cortex. GH (partly via insulin-like growth factor 1) (Møller and Jørgensen 2009), PRL (Freeman et al. 2000; Ben-Jonathan et al. 2006) and cortisol (Tsigos and Chrousos 2002) all exert effect on metabolism and the immune system, amongst many others functions. These hormones are secreted in pulsatile fashion and according to a circadian rhythm. The

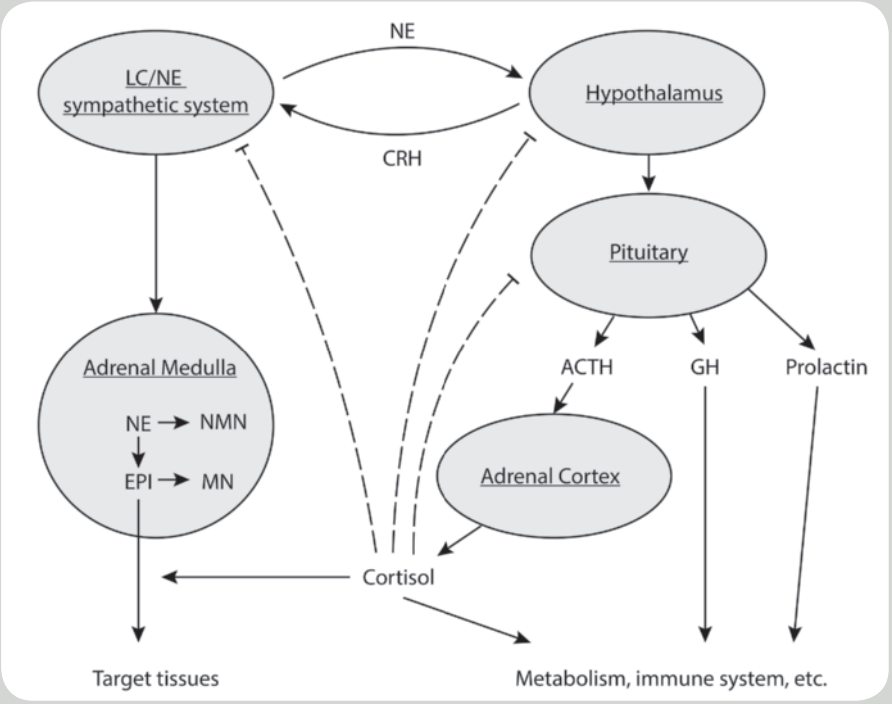


Figure 1.2. The pathways and interactions of the neuroendocrine and endocrine axes of the physiological stress system. Solid arrows: stimulation, Dashed capped lines: negative feedback, ACTH: adrenocorticotropic hormone, CRH: Corticotropin releasing hormone, EPI: epinephrine, GH: growth hormone, LC/NE: locus coeruleus noradrenergic system NE: norepinephrine, MN: metanephrine, NMN: normetanephrine. Adapted from Elenkov et al. (2000).

stress response is under self-regulating control by negative feedback loops (Elenkov et al. 2000; Tsigos and Chrousos 2002). That is, cortisol has an inhibitory effect via glucocorticoid receptors. These are omnipresent in the brain, including in the hypothalamus, the LC/NE sympathetic system and corticotrophic cells, and facilitate down-regulation of CRH and ACTH release. The neuroendocrine and endocrine axes of the stress response are intertwined both centrally and peripherally (Tsigos and Chrousos 2002).

Centrally, the hypothalamus and LC/NE sympathetic system stimulate each other via CRH and norepinephrine, respectively. Peripherally, cortisol mediates the effect of epinephrine on target organs by increasing the binding capacity of β-adrenergic receptors and reducing catecholamine reuptake.

Heart rate

$\dot{V}O_2$ is one of the most important determinants of endurance exercise capacity (Joyner and Coyle 2008). Heart rate is often used as its estimator, because of the linear relation between heart rate and $\dot{V}O_2$ for aerobic exercise. It should be noted, however, that besides the heart rate, also stroke volume and the arteriovenous oxygen difference determine $\dot{V}O_2$. Yet, in contrast to these determinants, heart rate measurements are easily applicable and widespread available due to the current technology. Hence, heart rate variables are often used to monitor training and recovery (Achten and Jeukendrup 2003; Bosquet et al. 2008; Halson 2014).

Choice reaction time

The overtraining spectrum, especially OTS, has been compared with major depression disorder (MDD) and the chronic fatigue syndrome (CFS) due to its symptomatic similarities (Armstrong and VanHeest 2002; Nederhof et al. 2006). Examples of shared symptoms are long-lasting feelings of fatigue, loss of vigour and motivation, and disturbed immune function. A slower reaction time has frequently been shown in MDD and CFS (Nederhof et al. 2006). Due to the resemblance between the overtraining spectrum and MDD and CFS, research on reaction time in the overtraining spectrum gained growing attention. Moreover, reaction time is inexpensive, objective, applicable in training practice, and not too demanding for athletes. It is, therefore, regarded as a potential tool for early detection of overtraining (Nederhof et al. 2006).
Two types of reaction time tests are distinguished, simple reaction time and

choice reaction time (Deary, Liewald, and Nissan 2011). Single reaction time tests measure the response time to a uniform stimulus, whereas choice reaction time tests require a suitable response to different stimuli. Both paradigms have been used in research on the overtraining spectrum. Single reaction time tests have been used at rest and during exercise (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010; Le Meur, Hausswirth, et al. 2013). Also several choice reaction time tests have been applied, such as the STROOP test (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010), Determination test (Nederhof et al. 2008), and the Finger-Precuing Task (Nederhof et al. 2007; Rietjens et al. 2005). The Finger-Precuing Task (FPT) requires a specific motor task (tapping with different fingers) to different stimuli. A cue signal before the stimulus determines the difficulty of the task. This test has been used frequently and showed promising results, and is therefore also applied in this thesis.

Monitoring of stressors and symptoms of overreaching

Stressors and early symptoms of overtraining are often monitored to detect an imbalance between stress and recovery. A survey among individuals working with athletes revealed that 91% implemented some form of training monitoring (Taylor et al. 2012). Yet, scientific data is limited because much of the information remains protected. Thus, most knowledge on training monitoring comes from anecdotal evidence and personal experience (Halson 2014; Bourdon et al. 2017).

The most important stressor related to overtraining is exercise. Exercise can be quantified by the external load, e.g. the distance or power output. Yet, monitoring of the internal training load is gaining popularity (Halson 2014). The internal training load is the physiological and psychological stress that results from exercise. This is often quantified by the session Rating of Perceived Exertion (Foster et al. 1995). Besides, several (online) monitoring systems have been developed that focus on the subjective response to training. An example is the SpartaNova training monitoring system (www.spartanova.

com). This tool includes daily ratings of sleep quality, mental and physical well-being, muscle soreness, fatigue, readiness to train, and attractiveness of the training day (Piacentini and Meeusen 2015).
One of the earliest symptoms of overtraining is mood disturbance (Halson et al. 2002). Mood can be quantified using the Profile of Mood States questionnaire (POMS). The POMS includes items on vigour, anger, fatigue, depression and tension, that are scored on a 5-point Likert scale (Wald and Mellenbergh 1990). Suggested physiological symptoms of increased training load are changes in resting heart rate and body temperature. Both can easily be measured and incorporated in daily routine, and thus have the potential to monitor the balance between (exercise) stress and recovery.

1.4 Tour for Life and the research design

The Tour for Life (TFL), the ecological source of this thesis, was expected to be a suitable experimental model, because 1) a heterogeneous group of amateur cyclists of various fitness levels participated in the event, 2) the increase in exercise volume was considerable, especially when compared to the regular activity level of the participants, and 3) this model allows measurements before, during and after the event in a relatively large study population. Therefore, this model provides a unique opportunity to study a heterogeneous response (AF and FOR) to increased training load. We defined the AF group as the group with no performance decrement and the FOR group as the group that underperformed after intensified training.
This research project was designed around the 2014 edition of the TFL, an 8-day non-competitive amateur cycling event. The TFL is a fundraising cycling tour from Bardonecchia, Italy, to Landgraaf, the Netherlands, covering 1264 km and 18,550 climbing meters (Figure 1.3). The subjects slept in small tents at camping sites. The start of the stages was between 0700h and 0800h. During the stage the participants took a few short breaks. The finish was between 1600h and 1930h, depending on the rider's fitness level and the

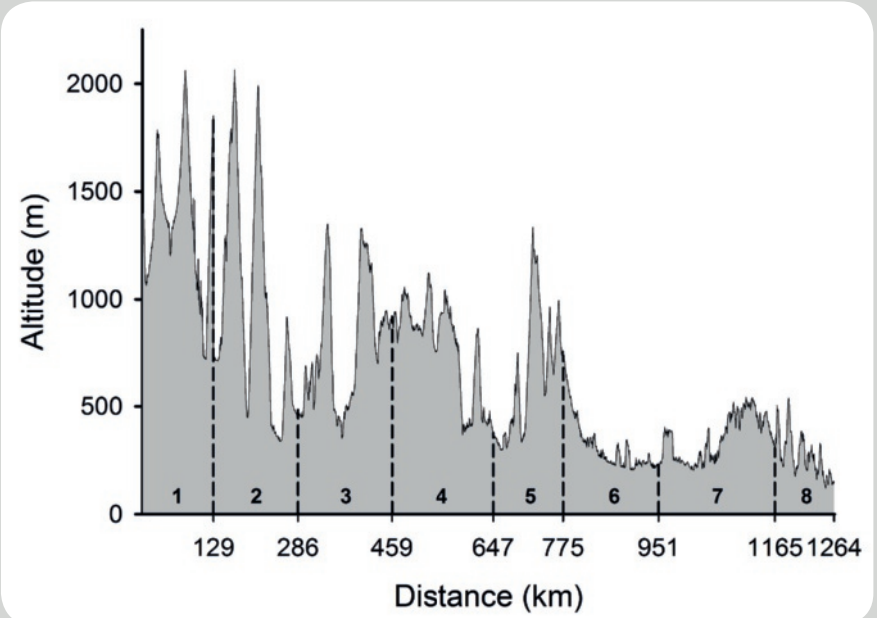


Figure 1.3. Altitude profile of the Tour for Life. Stage 1 to 8 are indicated with vertical dashed lines.

length of the stage. Most often the subjects rode the stage together with team members, which were of various fitness levels. The study design included field measurements (during the TFL) and well-controlled laboratory measurements before and after the event (Figure 1.4).

During the TFL, resting heart rate and rectal temperature were measured every morning. Within 30 minutes after the finish of stage 1 to stage 7 subjects reported the session Rating of Perceived Exertion (Foster et al. 1995) and completed a 7-item diary on subjective physical and mental well-being (Piacentini and Meeusen 2015). In addition to these daily measurements, data was collected at the start, middle and end of the TFL. This included overnight urine samples, and blood samples that were drawn before breakfast and immediately after the finish. The samples were directly processed in our mobile laboratory and frozen until further analyses. The 32-item Dutch

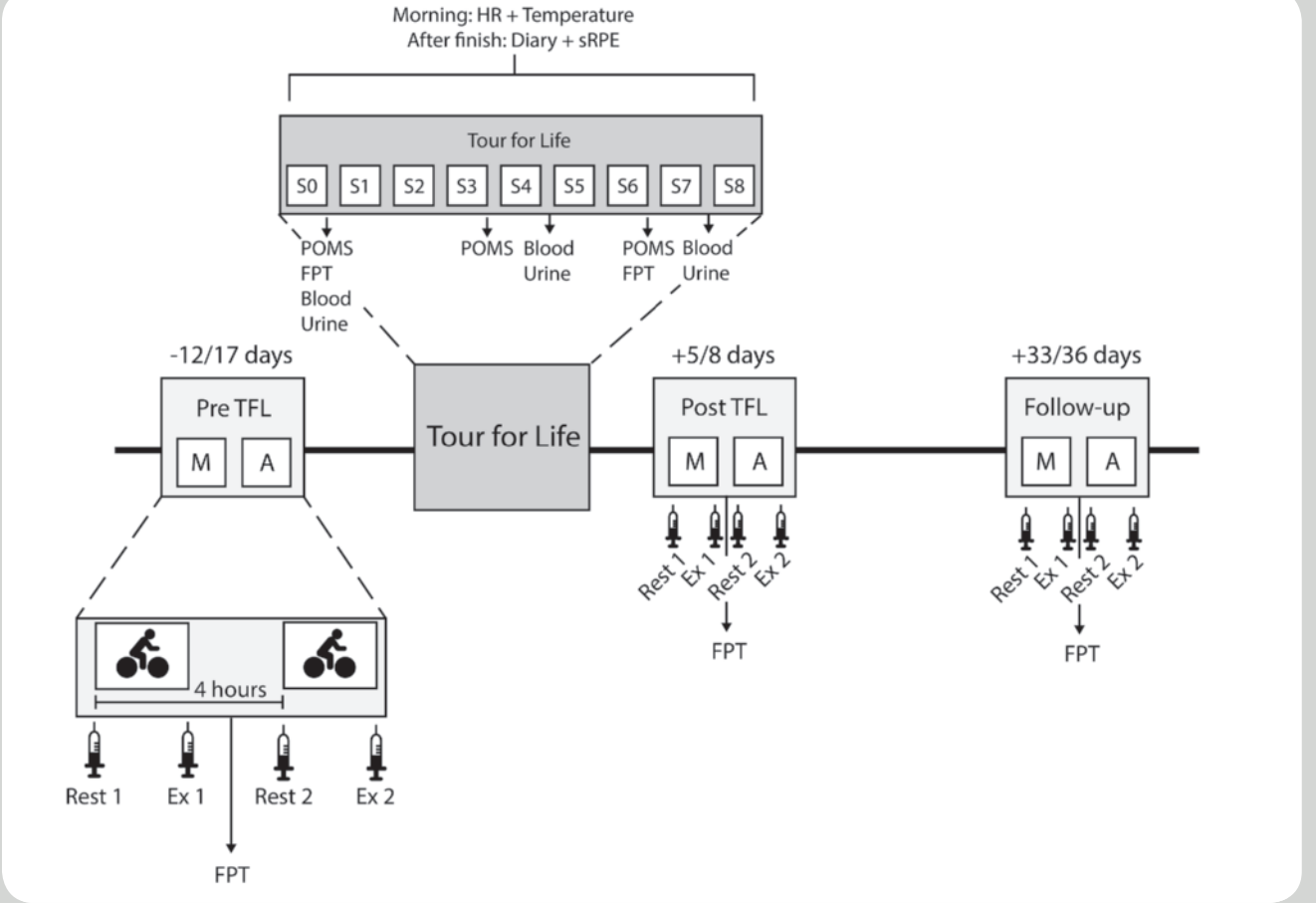


Figure 1.4. Research model. During the TFL, resting heart rate and rectal temperature were measured every morning, and session Rating of Perceived Exertion and a 7-item diary filled out after the finish of each stage (S0-S8). The Profile of Mood States questionnaire (POMS), Finger-Precuing Task (FPT) were applied, and blood and urine samples were collected at the start, halfway, and end of the TFL. Laboratory measurements were performed before and after the TFL. Each laboratory visit a maximal incremental exercise tests was performed in the morning (M), and 4 hours later in the afternoon (A). Blood samples were collected before (Rest) and after (Ex) each exercise test. The FPT was applied between the exercise tests.

version of the Profile of Mood States questionnaire (Wald and Mellenbergh 1990) was filled out and the Finger-Precuing Task (Adam et al. 1998) was applied.
Subjects visited the laboratory 12-17 days before the TFL (pre TFL), 5-8 days after the TFL (post TFL) and 33-36 days after the TFL (follow-up). Subjects were assigned to a timeslot, so that for each individual the pre TFL, post TFL and follow-up measurements were performed at the same time of day. Laboratory visits started at 0815h, 0930h and 1045h and lasted approximately 6 hours. Upon arrival at the laboratory the subjects handed in their overnight urine, body weight was measured, and the first blood sample was drawn (Rest 1). A portable ECG system (De Geus et al. 1995), was installed and worn the entire day. Then the morning maximal incremental cycling test was performed, after which a second blood sample (Ex 1) was drawn. Four hours after the start of the morning exercise test, the afternoon exercise test was performed. This means that the rest between both tests was about 3.5 hours, depending on the duration of the morning test. Again, blood samples were collected immediately before (Rest 2) and after (Ex 2) the exercise test. In the recovery period between both exercise tests the FPT was performed and lunch was provided.

1.5 Aim, hypotheses, and outline of the thesis

The aim of this thesis was to investigate whether hormonal levels, heart rate, reaction time, or monitoring of stressors and symptoms of overtraining can be used to distinguish between acute fatigue and functional overreaching. The parameters of interest will be addressed in chapter 2 to 6.

Chapter 2 – Hormonal levels during the Tour for Life
The overtraining spectrum, especially the most severe stages (NFOR/OTS), has been associated with changes in the (neuro)endocrine stress system (Barron et al. 1985; Meeusen et al. 2010; Cadegiani and Kater 2017b). Equivocal data

on resting hormonal levels in AF and FOR have been reported. In this chapter it was investigated whether ACTH, cortisol, growth hormone, prolactin, metanephrine and normetanephrine levels were altered during the Tour for Life. It was hypothesized that hormonal levels would change in FOR athletes, but not or to lesser extent in AF athletes.

Chapter 3 – Exercise-induced hormonal responses before and after the TFL
It has been suggested that endocrine disturbances related to the overtraining spectrum are most pronounced in response to a stimulus (Cadegiani and Kater 2017b). We applied a Two Bout Exercise Protocol (Meeusen et al. 2004) in our laboratory to examine exercise-induced hormonal levels before and after the Tour for Life. It was hypothesized that in FOR athletes 1) resting levels of ACTH, cortisol, GH and PRL would remain unchanged after the TFL, 2) exercise-induced hormonal responses would be reduced, most pronounced after the second exercise bout on a day, and 3) these changes would absent or less noticeable in AF athletes.

Chapter 4 – Heart rate during a maximal incremental cycling test
Heart rate is often used to prescribe and monitor intensified training. However, it is unknown whether the change in heart rate is associated with the result of intensified training, i.e. with the change in physical performance. In this chapter cardiopulmonary exercise tests were evaluated before and after the Tour for Life. It was hypothesized that (sub) maximal heart rate would be lower after this period of intensified training, but that this decrease would not be associated with a change in performance.

Chapter 5 – Choice reaction time
Data on reaction time in AF and FOR athletes is scarce, and inconsistent results have been reported. Therefore, previous studies concluded that additional research is necessary with more subjects and a relative large increase of exercise load (Halson 2014; Nederhof et al. 2007). Our TFL model fulfils

these requisites. In this chapter we applied the Finger-Precuing Task during and after the Tour for Life to investigate whether choice reaction time can be used to indicate FOR. It was hypothesized that reaction time would be slower in FOR, but not in AF athletes.

Chapter 6 – Monitoring of stressors and symptoms of overreaching
The subjective response to exercise, mood disturbances, resting heart rate and body temperature are easy, inexpensive, and quick measurements that can be used to monitor the effect of intensified training. In this chapter we combined the RPE, items from a validated online training monitoring system, the Profile of Mood States questionnaire (POMS), resting heart rate and rectal temperature to study which (combination of) parameters can be used to early distinguish between AF and FOR.

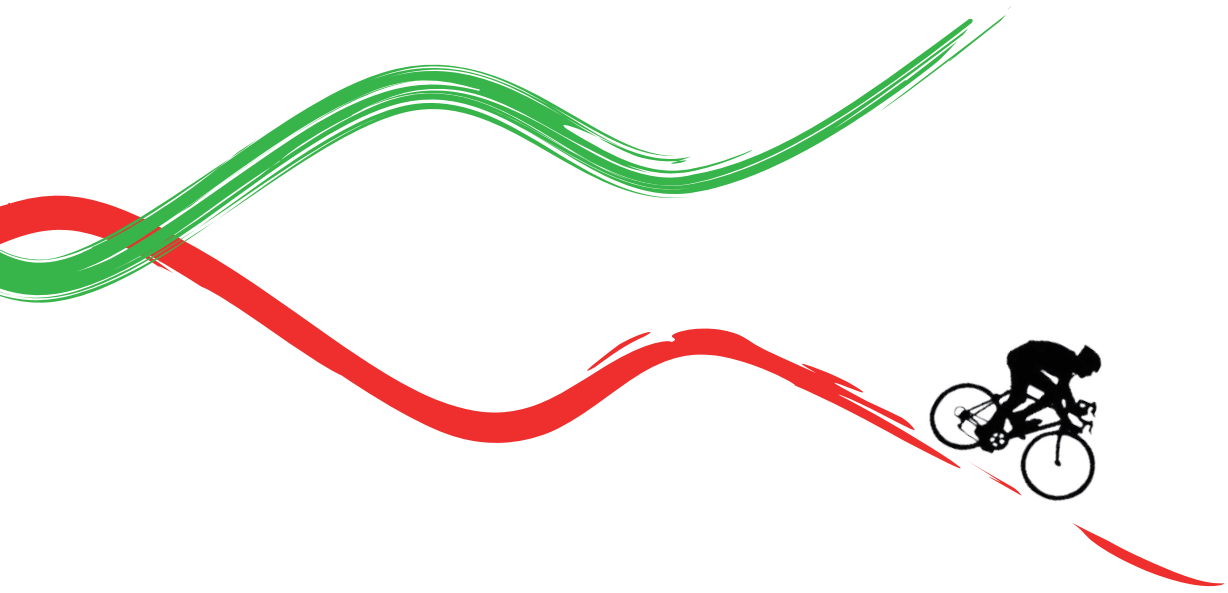
Chapter 7 – General discussion
The findings of this thesis are discussed from an integrative perspective. Moreover, directions for further research are provided, together with suggestions for applications of the results by professionals working with athletes.

Chapter 2

Hormonal changes in amateur athletes during an 8-day non-competitive cycling event

Twan ten Haaf • Selma van Staveren • Romain Meeusen • Bart Roelands • Maria Francesca Piacentini • Leo Koenderman • Carl Foster • Jos J. de Koning

Submitted for publication



Abstract

This study investigated whether hormonal changes during an 8-day non-competitive amateur cycling event (TFL) were different between athletes that underperformed after the TFL (FOR) and those who maintained their performance level (AF). Thirty cyclists (11 female/ 19 male, mean±SD $\dot{V}O2max$: 51.8±6.3 $ml \cdot kg^{-1} \cdot min^{-1}$, age: 40.8±10.8 years) were recruited. The TFL comprised 1300km, which was 9 fold the subjects' exercise volume during the preparation period. At the start, halfway and end of the TFL, blood samples were collected in the afternoon immediately after the stage and the following morning. Overnight urine samples were collected the same mornings. No differences between AF and FOR were observed in ACTH, cortisol, growth hormone, prolactin, metanephrine and normetanephrine (all P>.20). On total group level (AF+FOR), afternoon cortisol (P<.01) and growth hormone (P<.001) were increased from the start. Morning ACTH, cortisol and prolactin decreased, whereas GH increased during the TFL (all P<.001). Metanephrine and normetanephrine levels did not change significantly (both P=.08). End TFL growth hormone levels were most strongly associated (afternoon: r=-.68, morning: r=-.63) with the change in performance. This suggests that the observed changes in hormonal levels may be regarded a normal response to this high demanding cycling event, rather than maladaptation that results in underperformance (FOR).

2.1 Introduction

If an athlete repeatedly has insufficient time to recover between training bouts, the homeostasis of the physiological systems involved in exercise becomes disturbed. This multifactorial process is referred to as overtraining and can reflect itself in the possible outcomes of functional overreaching (FOR), non-functional overreaching (NFOR) or overtraining syndrome (OTS) (Meeusen et al. 2013). It has been suggested that the (neuro) endocrine stress response is one of the affected systems in the most severe stage of the overtraining continuum (i.e. OTS) (Barron et al. 1985; Lehmann et al. 1993). For example, recent studies showed reduced responsiveness of adrenocorticotropic hormone (ACTH), cortisol, growth hormone (GH) and prolactin (PRL) in OTS athletes compared to healthy athletes (Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). Other studies focused on changes on urinary catecholamines in OTS (Lehmann et al. 1992; Urhausen, Gabriel, and Kindermann 1998).

It is not clear whether the hormonal changes observed in OTS are present in FOR. A recent review suggested no changes in resting hormonal levels in FOR/NFOR induced athletes (Cadegiani and Kater 2017b), but some articles showed conflicting results. This probably results from a variety in sport modalities, a small number of studies, small sample sizes and/or a variety in FOR definitions. To illustrate the scarcity of data, 21 longitudinal studies were included in the review, while 55 other review articles were found (Cadegiani and Kater 2017b). The median sample size in these studies was 14. In our study we, therefore, recruited a relative large cohort of cyclists who participated in an 8-day non-competitive cycling event. Amateur cyclists of different fitness level, age and gender participated in this fundraising cycling tour. Nonetheless, the exercise overload was very big for all of them, and possibly resulted in underperformance after the event (i.e. FOR). The aim of this study was to examine whether hormonal levels changed during the 8-day event, and whether these changes were different for cyclists who underperformed after the event (FOR) than for cyclists who did not show a performance decrement.

Because hormonal changes possibly relate to the symptoms of FOR/NFOR/OTS, in particular underperformance, we hypothesized that hormonal levels changed in FOR athletes, but not or to lesser extent in athletes who were able to maintain or increase their performance level.

2.2 Methods

Subjects

Thirty amateur cyclists (11 female, 19 male) who participated in an 8-day non-competitive cycling event from Italy to the Netherlands (the Tour for Life, TFL) were recruited through the newsletter of the event. Healthy adults without injuries at the time of recruitment were included. At baseline, mean±SD $\dot{V}O_2$max was 51.8±6.3 ml·kg^{-1}·min^{-1}, peak power output 4.12±0.57 W·kg^{-1} and age 40.8±10.8 years. According to the $\dot{V}O_2$max-based athlete fitness

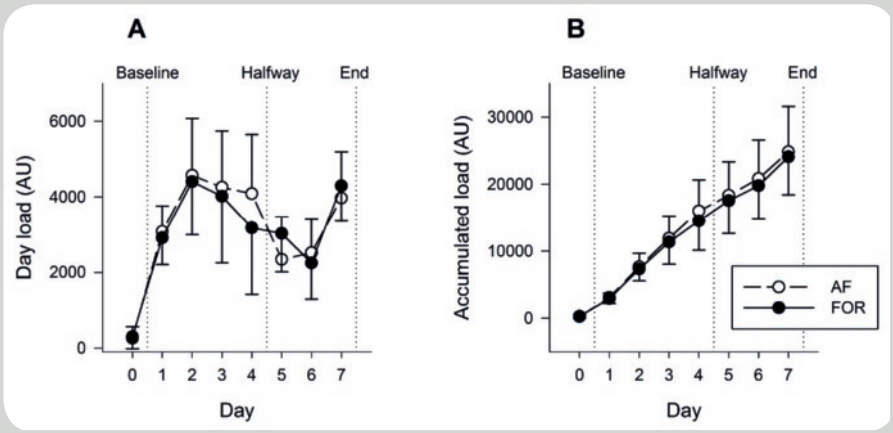


Figure 2.1. Daily (**A**) and accumulated (**B**) exercise load (RPE * duration) per stage of the TFL for athletes classified as acute fatigued (AF, open circles) and functionally overreached (FOR, filled circles). Timing of blood and urine sampling (baseline, halfway TFL, end TFL) are displayed with vertical dotted lines.

norms (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016) the subjects can be described as performance level 1 (4%), 2 (57%), 3 (25%) and 4 (14%). Before the first measurements, subjects gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical committee (VUmc 2014.319).

Design

The TFL is a non-competitive fundraising cycling event that comprised 8 consecutive days of cycling with a total of 1264 km and 18,550 climbing meters. Participants cycled on average 495±66 minutes per day and the Rating of Perceived Exertion was 6.9±1.2 out of 10. The exercise volume was 158±38 km per day, which was approximately 9 fold the exercise volume in the preparation period (ten Haaf et al. 2017). The daily and accumulated exercise load, calculated as the Rating of Perceived Exertion by exercise duration (Foster et al. 1995), are displayed in Figure 2.1.

Participants visited the laboratory 12-17 days before (pre), 5-8 days after (post) and 33-36 days after (follow-up) the TFL (Figure 2.2). During each laboratory visit the subjects performed two maximal incremental tests till exhaustion on a cycling ergometer (Meeusen et al. 2004). During the TFL, blood and nocturnal urine samples were collected before day 1 (start), after day 4 (halfway TFL) and after day 7 (end TFL). Blood samples were drawn in the afternoon immediately after the stage and the following morning after waking up.

Exercise tests

The laboratory exercise tests started with cycling (Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands, or; Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) at 80 W for 3 minutes. Power increased 40 W for men and 30 W for women every 3 minutes. The subjects cycled at

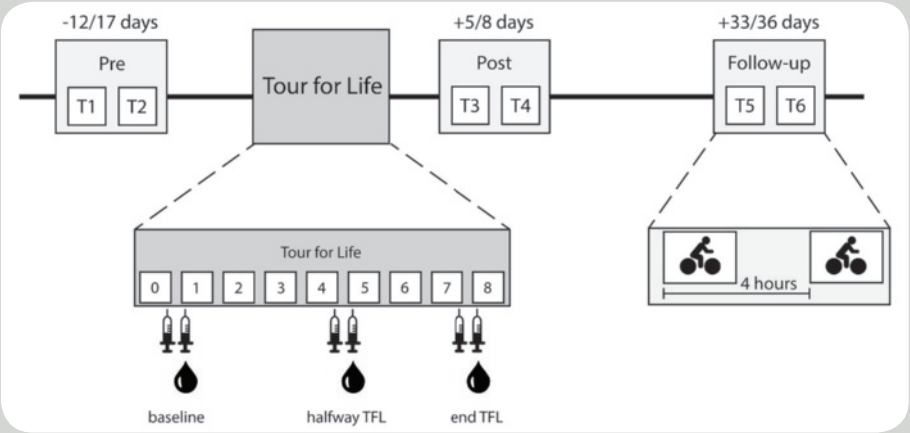


Figure 2.2. Study design. Incremental cycling ergometer tests (T) were performed before (pre), 1 week (post) and 5 weeks (follow-up) after an 8-day non-competitive amateur cycling event (Tour for Life). Afternoon and morning blood samples and nocturnal urine were collected at baseline, after day 4 (halfway TFL) and after day 7 (end TFL). The cycling stages of the event are indicated with numbers (1-8), 0 is the day before the first stage.

a freely chosen cadence. The test was stopped when subjects were unable to maintain the cadence above 60 rpm, despite strong verbal encouragement. Each participant performed the tests on the same ergometer and at the same time of the day. Saddle and handle bar height were measured after the first test and the same settings were used in subsequent tests. Peak power output was defined as the average power output over the last 3 minutes of the exercise test (i.e. PPO = Wcompl + Wincr * (t/180), where Wcompl is the last completed workload, Wincr is the increment in workload, and t is the number of seconds in the last workload). The physical performance for each visit was calculated as the average peak power output for the two exercise tests on the day.

Plasma ACTH

Peripheral blood was collected from the antecubital vein in

ethylenediaminetetraacetic acid (EDTA) and heparin containing vacutainers. Blood collected in the EDTA tubes was centrifuged 1500 g for 10 min and the plasma fraction was frozen at -30°C for maximally 8 days and then stored at -80 °C until further analysis. Plasma ACTH concentrations were measured using an electrochemiluminescence immunoassay on the Cobas E411 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The lower limit of detection was 2.0 $ng \cdot L^{-1}$ and inter-assay variation was 5.7 – 3.5% at 8.5 – 170 $ng \cdot L^{-1}$ respectively (N=18). Intra-assay variation was <1.2%. Normative values: morning measurements 5 - 70 $ng \cdot L^{-1}$; afternoon measurements < 50 $ng \cdot L^{-1}$.

Serum cortisol, growth hormone and prolactin

The heparin samples were immediately cooled in a refrigerator at 4 °C for at least 30 minutes and then spun in a refrigerated centrifuge at 1500 g for 10 minutes. Within 1 hour after the blood was drawn the samples were stored at -30 °C for maximally 8 days and then stored at -80 °C until further analysis. Serum concentrations of cortisol and PRL were determined by a chemiluminescence immunoassay on a Unicel Dxl 800 (Beckman Coulter, Bea, CA, USA). Inter-assay variation for cortisol was 5.4 – 8.2 % at 181 – 381 $\mu g \cdot L^{-1}$, respectively. Intra-assay variation was 3.4 – 6.2 % at 181 – 381 $\mu g \cdot L^{-1}$, respectively. Normative values: morning measurements 72.5-235.6 $\mu g \cdot L^{-1}$; afternoon measurements 36.2-126.9 $\mu g \cdot L^{-1}$. The lower limit of detection for PRL was 0.005 $IU \cdot L^{-1}$ and inter-assay variation was 1.42 – 1.61 % at 0.09 – 0.50 $IU \cdot L^{-1}$, respectively. Intra-assay variation 6.92 – 3.32 % at 0.09 – 0.50 $IU \cdot L^{-1}$, respectively (N=60). Normative values: female 0.02-0.53 $IU \cdot L^{-1}$; male 0.02-0.42 $IU \cdot L^{-1}$.

GH was measured using an immunometric technique on an IMMULITE 1000 analyser (Siemens Medical Solutions Diagnostics, Los Angeles, USA). The lower limit of detection was 0.15 $mIU \cdot L^{-1}$ and inter-assay variation was 7.4 – 3.7% at 0.42 – 24 $mIU \cdot L^{-1}$ respectively (N=70). Intra-assay variation

was <2.0% and the normative value: <20 mIU·L^{-1}.

Nocturnal urinary metanephrines

Nocturnal urine was collected from the moment subjects emptied their bladder before going to bed. Urine was collected during the night including the first morning urine. The collection jar contained 100 ml H_2SO_4 2N to prevent catecholamine degradation. The urine samples were analysed for metanephrine (MN) and normetanephrine (NMN), the more stable metabolites of adrenal epinephrine and norepinephrine, respectively (Eisenhofer et al., 1995). Analyses were performed using the 2-MET Urine ELISA kit BA E-8600 (LDN - Labor Diagnostika Nord, Nordhorn, Germany). The lower limit of detection was 0.1 µmol·L^{-1} for both parameters. Inter-assay variation was 14% at 0.9 µmol·L^{-1} for MN and 3.7% at 3.2 µmol·L^{-1} for NMN. Normative values MN: <1.8 µmol per 24hr; NMN <3.3 µmol per 24hr.

Classification: AF or FOR

Unexplained underperformance is one of the main symptoms of FOR, and the subjects in this study were classified accordingly. In line with previous research (Aubry et al. 2014; Decroix, Piacentini, et al. 2016; ten Haaf et al. 2017), subjects were classified as FOR if performance in both post TFL exercise tests decreased more than the smallest worthwhile change (i.e. T1-T3>SWC*T1 & T2-T4<SWC*T2, Figure 2.2). All other subjects were classified as acute fatigued (AF). The smallest worthwhile change was calculated as 0.3 * the coefficient of variation of peak power output between the 2 exercise tests pre TFL (T1 and T2 in Figure 2.2) (Hopkins, Hawley, and Burke 1999).

Statistical analyses

Normality was checked using Shapiro-Wilks test and non-normal data was

log-transformed. Mixed models were used to analyse ACTH, cortisol, GH, PRL, MN and NMN. Time (start, halfway TFL, end TFL) was included as within-subject factor and classification (AF, FOR) as between-subject factor. Sex was included as covariate since sex differences have been observed in research on the hormonal stress response (Uhart et al. 2006). Because it is impossible to distinguish between acute and accumulated fatigue, values halfway and at the end of the TFL cannot be compared. Therefore, significant main effects were followed-up by Bonferroni adjusted planned comparisons between start and halfway TFL or start and end TFL, but not between halfway TFL and end TFL. Main effects and interactions were considered significant if $P<.05$. Hormonal changes in terms of absolute numbers and percentage are expressed as median values.

In addition to dichotomized statistical testing (i.e. AF versus FOR), Pearson's correlation coefficients were calculated to investigate relations between changes in hormone levels during the TFL (start versus halfway TFL; and start versus end TFL) and changes in physical performance as a result of the cycling event (pre versus post TFL; and pre TFL versus follow-up). All analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0, Amonk, NY).

Table 2.1. Subject characteristics (mean±SD).

	AF (N=14)	**FOR (N=15)**	**P-value**
Sex (m/f)	11/3	7/8	.13
Age (yrs)	42.6 ± 11.0	39.1 ± 10.7	.39
Body mass index ($kg \cdot m^{-2}$)	24.0 ± 1.7	23.0 ± 2.3	.22
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.3 ± 6.2	51.8 ± 6.6	.83
Peak power ($W \cdot kg^{-1}$)	4.15 ± 0.61	4.06 ± 0.52	.64

AF: acute fatigued, FOR: functional overreached

2.3 Results

Subjects

One participant was excluded from analyses because of asthmatic symptoms during the post TFL exercise tests. No blood (ACTH, cortisol, GH, PRL) was available for one other participant in the afternoon halfway through the TFL. The coefficient of variation of the exercise test pre TFL was 1.6%, meaning the smallest worthwhile change was 0.5%. According to the AF/FOR criteria, 14 subjects were classified as AF and 15 as FOR. The baseline characteristics of the subjects are shown in Table 2.1.

Hormones

None of the afternoon hormonal levels showed a significant between-subject effect for classification (ACTH: P=.71; cortisol: P=.27; GH: P=.20; PRL: P=.66) or a time by classification interaction effect (ACTH: P=.94; cortisol: P=.55; GH: P=.46; PRL: P=.76). For the total group (AF+FOR), afternoon cortisol levels increased during the TFL (Figure 2.3B). Halfway TFL, the median increase was 9.1 $\mu g \cdot L^{-1}$ (median: 15%, P=.03), and at end TFL cortisol was 19.9 $\mu g \cdot L^{-1}$ higher than at the start (31%, P<.01). Similarly, GH halfway TFL was 5.44 $mIU \cdot L^{-1}$ (825%, P<.001), and at end TFL 2.66 $mIU \cdot L^{-1}$ (635%, P<.001) higher than at the start (Figure 2.3C). ACTH (P=.52) and PRL (P=.09) did not change during the TFL.

Similar as for the afternoon levels, no significant effect for classification (ACTH: P=.33; cortisol: P=.44; GH: P=.38; PRL: P=.18; MN: P=.73; NMN: P=.64) or a time by classification interaction effect (ACTH: P=.17; cortisol: P=.53; GH: P=.81; PRL: P=.89; MN: P=.40; NMN: P=.21) was observed in the morning hormonal levels. Yet, a main effect of time (AF+FOR) was found for the blood derived hormones (Figure 2.4). ACTH, cortisol and PRL decreased, whereas GH increased during the TFL. The median ACTH decrease halfway TFL was -18 $ng \cdot L^{-1}$ (median: -31%, P<.001) and at end

TFL -26.5 ng·L^{-1} (-50%, P<.001) compared to the start. PRL decreased -0.06 IU·L^{-1} (-21%, P=.046) and -0.13 IU·L^{-1} (-37%, P<.001) halfway TFL and end TFL, respectively, whereas cortisol was only at end TFL lower than at the start (-18.1 µg·L^{-1}, -11%, P<.01). In contrast, GH was higher both halfway TFL (1.61 mIU·L^{-1}, 232%, P<.001) and at the end TFL (1.16 mIU·L^{-1}, 336%, P<.001) than at the start. No significant changes in metanephrine (P=.08) and normetanephrine (P=.08) were observed. Median values and the interquartile range for AF and FOR at the start, halfway TFL and end TFL are provided in Table 2.2 and Table 2.3.

Association hormones and physical performance

The Pearson's correlation coefficients showed that the change in performance 1 week after the TFL (post TFL) was strongest related to the change in GH (Table 2.3). The negative relations revealed that a bigger decrease in performance was associated with a higher increase in GH in the morning (halfway versus start TFL: *r*=-.55, P<.01; end TFL versus start: *r*=-.63, P<.001) and afternoon samples (end versus start TFL: *r*=-.68, P<.001). Weaker positive relations were found for morning ACHT (halfway versus start TFL: *r*=.38, P=.04; end versus start TFL: *r*=.38, P=.04). Also the change in PRL halfway TFL was associated with the change in pre versus post TFL performance (afternoon: *r*=-.61, P<.001; morning: *r*=.47, P=.01).

The relations between the change in performance at follow-up and hormonal changes during the TFL were less pronounced. Only the decrease in morning cortisol (*r*=.42, P=.03) and increase in MN (*r*=-.39, P=.04) and NMN (*r*=-.40, P=.04) at the end of the TFL were weakly associated with a decrease in performance at follow-up compared to pre TFL.

2.4 Discussion

The primary aim of this study was to examine whether amateur athletes who

Table 2.2. Afternoon hormonal levels for athletes classified as acute fatigued (AF) and functionally overreached (FOR) at baseline, halfway TFL and end TFL. Values are displayed as median [25th; 75th percentile].

	Baseline		Halfway TFL		End TFL	
	AF	**FOR**	**AF**	**FOR**	**AF**	**FOR**
ACTH ($ng \cdot L^{-1}$)	20 [11; 25]	15 [12; 23]	17 [9; 22]	14 [12; 22]	17 [10; 24]	15 [13; 26]
Cortisol ($\mu g \cdot L^{-1}$)	67.1 [56.2; 101.5]	47.1 [39.9; 76.1]	77.9 * [65.2; 105.1]	77.9 * [63.4; 94.2]	85.2 * [72.5; 102.4]	88.8 * [62.5; 126.9]
GH ($mIU \cdot L^{-1}$)	0.24 [0.16; 3.40]	0.69 [0.28; 1.68]	2.95 * [1.55; 2.95]	7.30 * [4.58; 10.15]	2.30 * [0.93; 4.00]	5.75 * [3.43; 10.55]
Prolactin ($IU \cdot L^{-1}$)	0.13 [0.12; 0.21]	0.15 [0.09; 0.20]	0.18 [0.13; 0.22]	0.14 [0.10; 0.31]	0.19 [0.13; 0.27]	0.17 [0.15; 0.27]

ACTH: adrenocorticotropic hormone, GH: growth hormone, AF: acute fatigue, FOR: functional overreaching.

* Significantly different from baseline (P<.05)

Table 2.3. Morning hormonal levels for athletes classified as acute fatigued (AF) and functionally overreached (FOR) at baseline, halfway TFL and end TFL. Values are displayed as median [25th; 75th percentile].

	Baseline		Halfway TFL		End TFL	
	AF	**FOR**	**AF**	**FOR**	**AF**	**FOR**
ACTH ($ng \cdot L^{-1}$)	52 [43; 70]	71 [42; 94]	41 * [31; 54]	45 * [31; 54]	31 * [24; 35]	22 * [15; 37]
Cortisol ($\mu g \cdot L^{-1}$)	181.2 [173.1; 193.0]	170.4 [157.7; 210.2]	190.3 [151.3; 217.5]	183.0 [169.4; 195.7]	159.5 * [139.5; 185.8]	148.6 * [134.1; 173.1]
GH ($mIU \cdot L^{-1}$)	0.49 [0.25; 1.55]	0.73 [0.25; 4.15]	2.15 * [0.76; 6.15]	2.80 * [0.62; 23.23]	2.45 * [0.68; 9.38]	3.90 * [0.78; 17.23]
Prolactin ($IU \cdot L^{-1}$)	0.37 [0.31; 0.41]	0.35 [0.26; 0.55]	0.29 * [0.19; 0.45]	0.27 * [0.21; 0.38]	0.22 * [0.17; 0.27]	0.20 * [0.14; 0.32]
MN (μmol)	0.11 [0.07; 0.15]	0.09 [0.04; 0.18]	0.10 [0.04; 0.26]	0.09 [0.05; 0.25]	0.13 [0.03; 0.36]	0.13 [0.10; 0.41]
NMN (μmol)	0.67 [0.36; 0.99]	0.62 [0.32; 97]	0.51 [0.32; 1.21]	0.49 [0.24; 0.84]	0.63 [0.25; 1.56]	0.78 [0.52; 1.74]

ACTH: adrenocorticotropic hormone, GH: growth hormone, MN: metanephrine, NMN: normetanephrine, AF: acute fatigue, FOR: functional overreaching.

* Significantly different from baseline (P<.05)

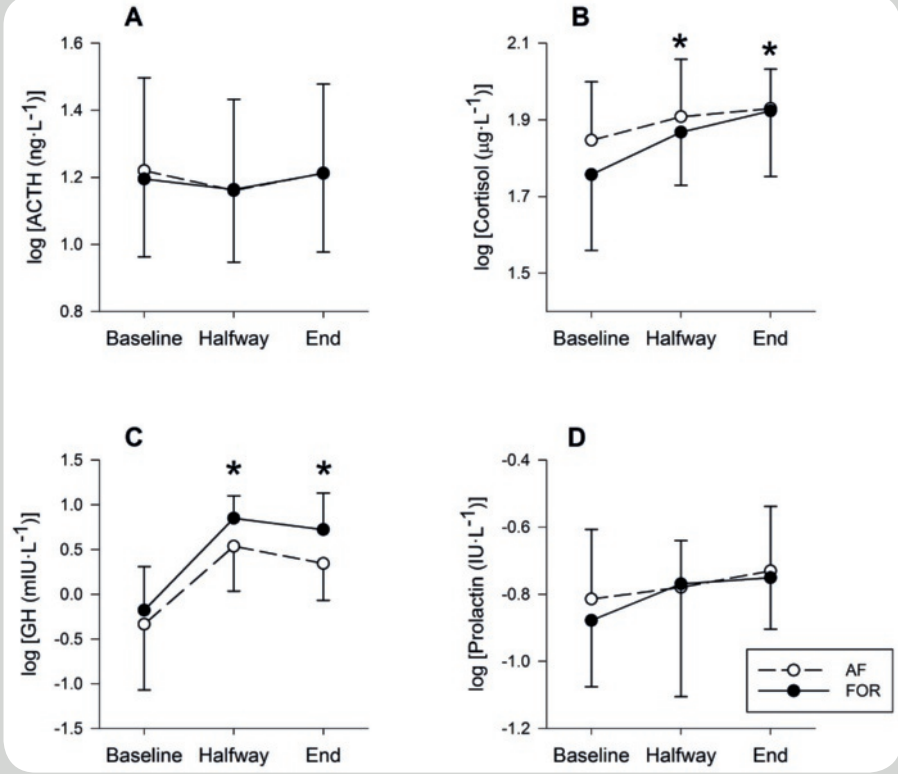


Figure 2.3. Afternoon levels of adrenocorticotropic hormone (**A**), cortisol (**B**), growth hormone (**C**) and prolactin (**D**) for athletes that were acute fatigued (AF, open circles) and functionally overreached (FOR, filled circles) at baseline, halfway TFL and end TFL. Mean±SD of the log transformed data is displayed because the data were non-normally distributed. * indicates significantly different from baseline (P<.05).

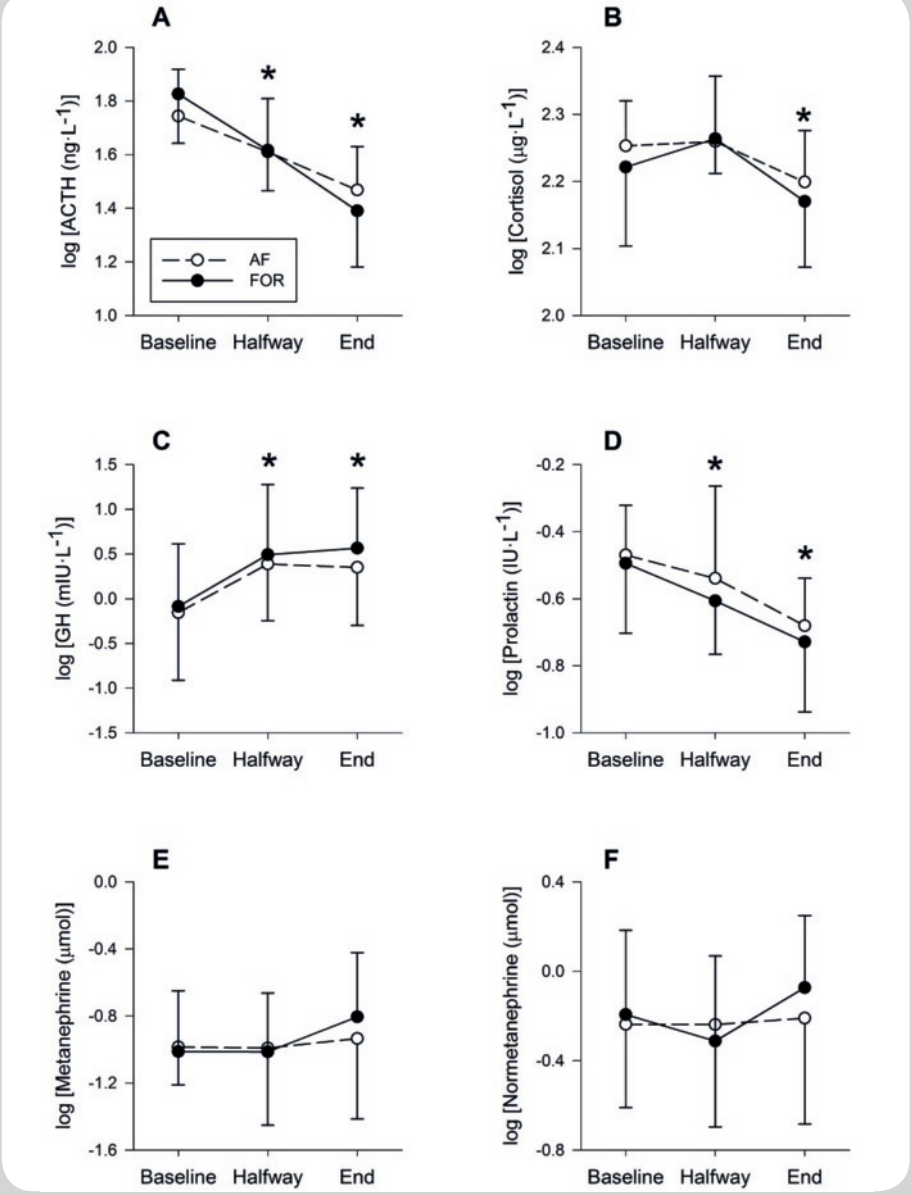


Figure 2.4. Morning levels of adrenocorticotropic hormone (**A**), cortisol (**B**), growth hormone (**C**), prolactin (**D**), metanephrine (**E**) and normetanephrine (**F**) for athletes that were acute fatigued (AF, open circles) and functionally overreached (FOR, filled circles) at baseline, halfway TFL and end TFL. Mean±SD of the log transformed data is displayed because the data were non-normally distributed. * indicates significantly different from baseline (P<.05).

Table 2.4. Pearson's correlation coefficients (N=29) for the associations between hormonal changes during the TFL (baseline vs. halfway TFL; and baseline vs. end TFL) and change in performance as a result of the TFL (pre vs. post TFL; and pre TFL vs. follow-up).

	ΔPerformance (pre vs post TFL)		ΔPerformance (pre vs follow-up)	
	ΔHormone halfway TFL	ΔHormone end TFL	ΔHormone halfway TFL	ΔHormone end TFL
Afternoon				
ΔACTH (ng·L^{-1})	-.10	-.04	-.20	-.17
ΔCortisol (µg·L^{-1})	-.06	-.13	.02	.05
ΔGH (mIU·L^{-1})	-.27	**-.68****	-.23	-.12
ΔProlactin (IU·L^{-1})	**-.61****	.05	.14	.17
Morning				
ΔACTH (ng·L^{-1})	**.38***	**.38***	.26	.23
ΔCortisol (µg·L^{-1})	.17	.16	.33	**.42***
ΔGH (mIU·L^{-1})	**-.55****	**-.63****	-.32	-.26
ΔProlactin (IU·L^{-1})	**.47***	.12	.08	-.28
ΔMN (µmol)	.30	.10	-.36	**-.39***
ΔNMN (µmol)	.24	.31	-.35	**-.40***

ACTH: adrenocorticotropic hormone, GH: growth hormone, MN: metanephrine, NMN: normetanephrine

*Significant at P<.05; ** significant at P<.01

underperformed (FOR) after an 8-day non-competitive cycling event showed a different hormonal profile during the event than cyclists who maintained their performance level (AF). The results of this study indicate that hormonal levels changed during the TFL, but without differences between AF and FOR athletes. This suggests that the observed changes in hormonal levels may be regarded as a normal response to this high demanding cycling event, rather than maladaptation that results in underperformance (FOR). In accordance

with previous studies (Aubry et al. 2014; Decroix, Piacentini, et al. 2016; ten Haaf et al. 2017), classification of AF/FOR in our study was based on the smallest worthwhile change. The smallest worthwhile change in our study was a -0.5% decrease in peak power output during the post TFL exercise tests. This was comparable to -0.6% as shown in the previous studies using this criteria (Aubry et al. 2014; Aubry et al. 2015).

On total group level (AF+FOR), GH stands out with an increase in both afternoon and morning levels, whereas most hormones decreased during the TFL. Our observation of increased GH concentrations is in line with Lehmann et al. (1998), who suggested that GH release may be increased in the early stage and decreased in the more severe stage of the overtraining continuum. Yet, other studies found no significant changes in GH concentrations after a period of intensified training (Lehmann et al. 1993; Meeusen et al. 2004; Rietjens et al. 2005). A remarkable difference between these studies and our study is that the exercise overload in our study (~ 9 fold) exceeded the other studies (up to ~ 2 fold). Importantly, in none of the other studies did the applied overload result in underperformance. This illustrates that the physical challenge in our study was much bigger. GH is a metabolic master-switch with effects on metabolism and protein synthesis (Møller and Jørgensen 2009). In periods of energy deficit the main effect of GH is to increase lipid mobilization and oxidation. Its effect on protein metabolism during these periods of fuel shortage is not straightforward. The oxidation of fat might partly function as a mechanism to prevent protein being utilized as an energy source. When nutritional intake is sufficient, GH stimulates the production of IGF-1 in the liver (Møller and Jørgensen 2009). IGF-1 is a crucial mediator of the protein anabolic effect of GH. Only GH was associated with changes in physical performance 1 week after the TFL (post TFL), but not with performance change 5 weeks after the TFL (follow-up). These findings support the hypothesis of Steinacker et al. (2004) that metabolic stress and/or muscle damage are the main cause of short-term changes in physical performance. Due to the nature of our study model the energy balance of the

subjects was not controlled. Therefore, it is unclear whether the elevated GH levels in our study originated from metabolic demand and/or need for protein synthesis. Hecksteden et al. (2016) showed in a recent study that the decrease in IGF-1 and increase in urea were the best indicators for fatigue in 28 cyclists after 6 days of overload training. These findings indicate increased protein turnover and an energy deficit. Therefore, it is tempting to speculate that the primary cause of elevated GH levels and decreased physical performance in our study was an energy intake that was insufficient to match the high metabolic demands of the cycling event.

MN and NMN showed a trend towards increase, but no statistical significant differences were observed (P=.08). Comparable results were shown by Filaire et al. (2004), who reported significant increased NMN and a trend towards elevated MN levels in nocturnal urine after 4 days of intensified training. Remarkably, changes in MN and NMN were, together with cortisol, the only markers that showed an association with the long lasting change in performance (i.e. at follow-up). This means that a bigger increase in nocturnal urinary metanephrines during the TFL was related to underperformance at follow-up. Physiological interpretation of this finding is difficult because the exercise load between the TFL and the follow-up exercise test was not controlled. Due to the natural setting of the study, the follow-up measurement was performed near the end of the Dutch cycling season. Some subjects continued training whereas others did not cycle in this period. The change in performance at follow-up as a result of overreaching can therefore not be distinguished from detraining.

A down-regulation compared to the start of the TFL was observed for ACTH, cortisol and PRL in the morning samples at the end of the TFL. Only a few studies reported resting PRL levels after overload training (Lehmann et al. 1992; Meeusen et al. 2004; Steinacker et al. 2000). None of these showed statistical significant differences, although the decrease in two of the studies (~ -22% and ~ -25%) was comparable to our study (halfway TFL: -21%, end TFL: -37%). Our finding that morning ACTH decreased from the start of

the TFL is in line with previous studies (Hecksteden et al. 2016; Svendsen et al. 2016), but not confirmed by others (Coutts et al. 2007; Rietjens et al. 2005). In our study, cortisol was unaltered halfway through the TFL, but a decrease at the end compared to the start was observed. Most studies did not find changes in resting cortisol concentrations in the blood after intensified training (Hasegawa et al. 2015; Hecksteden et al. 2016; Meeusen et al. 2004; Piacentini et al. 2016; Urhausen, Gabriel, and Kindermann 1998), although exceptions found a decrease (Hedelin et al. 2000) or an increase (Svendsen et al. 2016). It seems that cortisol levels were relatively stable and only changed after prolonged endurance exercise, whereas ACTH was already reduced after a few days of cycling.

Chronic fatigue syndrome and burn-out syndrome are examples of pathological states in which lasting stress eventually results in paradoxically low cortisol levels (Heim, Ehlert, and Hellhammer 2000). Both chronic stress-related disorders show symptomatic similarities with FOR and the OTS (Meeusen et al. 2013), and the comparison between burn-out and overtraining has been made in previous studies (Nederhof et al. 2006; Armstrong and VanHeest 2002). In research on the chronic fatigue syndrome and burn-out, three mechanisms have been proposed that can explain lower cortisol concentrations after a period of chronically elevated levels of cortisol: 1) Reduced biosynthesis or depletion of CRH, ACTH and/or cortisol; 2) Increased negative feedback sensitivity to cortisol; 3) Down-regulation of specific receptors on different levels of the axis, i.e. the hypothalamus, pituitary, adrenals, and/or targets cells (Fries et al. 2005). We did not apply any direct measure in our study that can give insight in these three mechanisms. An interesting direction for further research is to investigate the (change in) sensitivity of the negative feedback pathway and the possible down-regulation of corticotrophic cell receptor sensitivity.

This field study gave us the opportunity to investigate changes in several hormones simultaneously during a non-competitive cycling event that resulted in overreaching in part of subjects. However, the naturally experimental

character of the study also implies some limitations. Firstly, it is impossible to distinguish between acute and accumulated fatigue. Especially for the afternoon measurements the varying daily exercise loads made it impossible to compare values halfway with the end of the TFL. Secondly, hormonal concentrations were determined at rest instead of in responses to a stimulus. Therefore, the circadian rhythm and pulsatile secretion of these hormones may hinder interpretation. However, it is unlikely that the circadian rhythm caused the observed changes in hormone levels. The samples halfway TFL and end TFL were drawn 105±47 minutes later than at the start of the TFL (the difference between the morning samples was only 10±47 minutes, data not shown). The circadian rhythms of ACTH and cortisol suggest lower concentrations later in the afternoon. This means that the effect of the circadian rhythm, if any, resulted in an underestimation of the increase in afternoon cortisol. Moreover, it is implausible that pulsatile secretion accounted for the observed differences. The decrease in morning ACTH, cortisol and PRL and increase in GH was observed in 97%, 73%, 90% and 83% of the subjects, respectively. This strong pattern is unlikely the result of pulsatile hormonal secretion. Thirdly, the effect of hormones on target cells is not only determined by hormonal concentrations. Target cell receptor density and sensitivity, inactivation by binding molecules and pre-receptor metabolism by tissue specific enzymes can all play a role in the exerted physiological effect of hormones (Duclos, Guinot, and Le Bouc 2007). For example, the effect of catecholamines depends on adrenergic receptor density and sensitivity, which decrease with repeated endurance exercise (Lehmann et al. 1998).

2.5 Conclusion

The results of this study showed that hormonal levels changed in amateur athletes during an 8-day non-competitive cycling event with 9 fold increase in exercise load. However, no differences were observed in the hormonal profile between AF and FOR athletes. This suggests that the observed changes may

be regarded normal adaptations in the acute fatigue/functional overreaching stage of the overtraining continuum, rather than a determining factor of underperformance.

Chapter 3

Reduced exercise-induced ACTH, cortisol and prolactin responses after an 8-day non-competitive amateur cycling event

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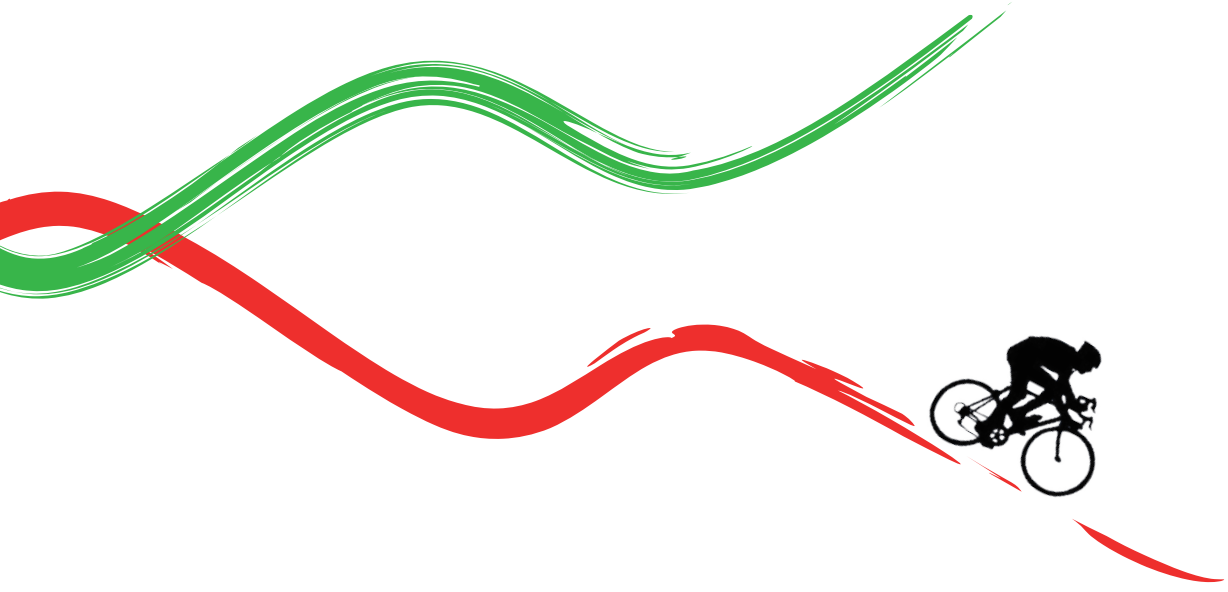
Submitted for publication



Abstract

The purpose of this study was to investigate whether adrenocorticotropic hormone (ACTH), cortisol, growth hormone (GH) and prolactin (PRL) responses to an exercise test changed after an 8-day non-competitive amateur cycling event (TFL). In addition, it was investigated whether hormonal responses were different between athletes who underperformed after the TFL (FOR) and those who maintained their performance level (AF). Thirty subjects were recruited with an average age of 40.8±10.8 y and $\dot{V}O_2$max of 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. Two weeks before (pre), 1 week after (post) and 5 weeks after (follow-up) the TFL, subjects performed a maximal incremental cycling test in the morning and afternoon. Blood was drawn before (Rest) and after (Ex) each test. It was shown that resting ACTH (P<.01) and GH (P<.001) were higher pre than post TFL. The response to the morning test was higher pre than post TFL for ACTH (P=.02), cortisol (P<.001) and PRL (P<.01), but not for GH (P=.12). The response to the afternoon test was only for cortisol higher pre than post TFL (P<.001). None of the observed effects were different between AF and FOR. In conclusion, exercise-induced ACTH, cortisol and PRL responses were reduced after an 8-day non-competitive amateur cycling event. Changes were mainly found for the morning, rather than for the afternoon exercise test. The observed hormonal changes were not related to underperformance (FOR).

3.1 Introduction

Functional overreaching (FOR) results from an imbalance between exercise and recovery (Meeusen et al. 2013). FOR is characterized by a short-term decrease in physical performance (days to weeks), possibly accompanied by other complaints such as mood and sleep disturbances and reduced immune function. If athletes continue their imbalanced training regimen, a state of non-functional overreaching (NFOR) or eventually overtraining syndrome (OTS) may develop. NFOR and OTS are the most severe stages of the overtraining spectrum and take weeks to months, and months to years to recover, respectively.

The endocrine system is one of the systems likely affected in the overtraining spectrum (Cadegiani and Kater 2017b; Meeusen et al. 2013). In research on the overtraining spectrum, hormones are most often measured at rest. However, a recent review suggested that resting hormonal levels remain unchanged, while hormonal responses to a stimulus are altered (Cadegiani and Kater 2017b). It has been shown that the responses of adrenocorticotropic hormone (ACTH), cortisol, growth hormone (GH) and prolactin (PRL) to an insulin tolerance test were smaller in OTS athletes than in healthy athletes, but not different from sedentary controls (Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). It was suggested that OTS athletes lost the positive endocrine adaptations seen in healthy athletes, which might contribute to the loss of physical performance in OTS. It is, however, not clear whether the observed hormonal changes in OTS could be found earlier in the overtraining spectrum (i.e. FOR).

Previous research applied a two bout exercise protocol (TBEP) to study the exercise-induced hormonal response in overtraining (Meeusen et al. 2004; Meeusen et al. 2010). The TBEP is inspired by the observation that OTS athletes have difficulties with performing a second exercise session on a day. The protocol, sometimes extended with measurements on mood and reaction time and referred to as the Training OPtimisation test (TOP-test), has also been used to predict drop-outs of harsh military training (Vrijkotte et al.

2017). In NFOR and OTS athletes it was observed that altered hormonal responses were most noticeable after a second exercise bout on the same day. Consequently, it was proposed that the recovery capacity of the endocrine system is affected in overtraining.

The aims of this study were to investigate whether ACTH, cortisol, GH and PRL changed after a short period of imbalanced exercise and recovery, and whether hormonal adaptations are related to the change in performance. This was examined by investigating the hormonal response to the TBEP before and after an 8-day non-competitive amateur cycling event that possibly resulted in FOR. It was hypothesized that 1) resting levels of ACTH, cortisol, GH and PRL would remain unchanged, 2) exercise-induced hormonal responses would be reduced after the event, most noticeable after the second exercise bout on one day, and 3) reduced hormonal responses would be found in cyclists that underperformed after the event (i.e. FOR), but not or less in subjects who maintained their performance level.

3.2 Methods

Subjects

Thirty healthy amateur cyclists without known injuries were recruited for this study (11 female, 19 male). At baseline, $\dot{V}O_2$max was (mean±SD) 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$, peak power output was 4.12±0.57 $W \cdot kg^{-1}$ and age was 40.8±10.8 y. The subjects were of performance level 1 (4%), 2 (57%), 3 (25%) and 4 (14%) according to $\dot{V}O_2$max-based athlete classification norms (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016). Before the first measurements, subjects gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical committee (VUmc 2014.319).

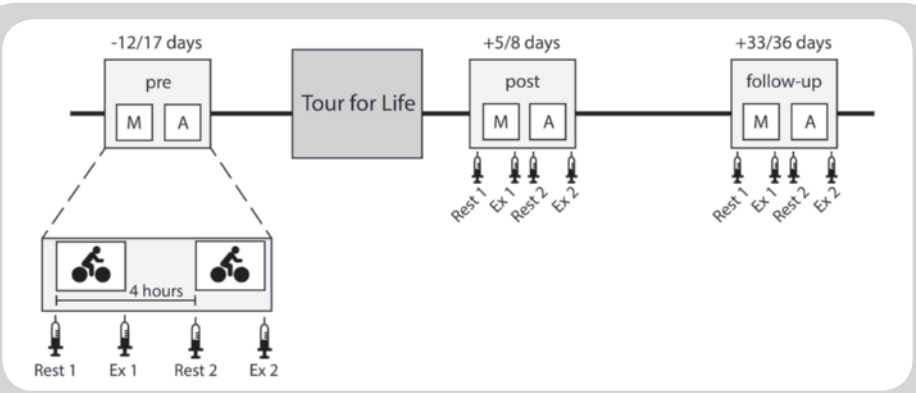


Figure 3.1. Study design. The 2 bout exercise protocol (TBEP) was performed 2 weeks before (pre) and 1 (post) and 5 (follow-up) weeks after the Tour for Life. The TBEP consists of a morning (M) and afternoon (A) incremental cycling ergometer tests to exhaustion, seperated by 4 hours. Blood was drawn before (Rest) and after (Ex) all exercise bouts.

Design

The TBEP was applied 12-17 days before (pre), 5-8 days after (post) and 33-36 days after (follow-up) an 8-day non-competitive amateur cycling event (TFL) (Figure 3.1). The TFL comprised 8 consecutive days of cycling with a total of 1264 km and 18,550 vertical climbing meters. It was reported previously that subjects cycled on average 495±66 minutes per day with a session Rating of Perceived Exertion (Foster et al. 1995) of 6.9±1.2 on a 0-10 visual analogue scale (ten Haaf et al. 2017). The exercise volume (distance) was approximately 9 fold compared to the volume during the preparation period.

The pre TFL, post TFL and follow-up laboratory visits for each subject started at the same time of day, by randomly assigning subjects to a timeslot (start measurements at 0815h, 0930h or 1045h each visit). Subjects did not perform vigorous exercise within 36 hours, and refrained from alcohol within 24 hours prior to the laboratory visits. Habitual breakfast was consumed at home, at least 2 hours before the first laboratory exercise test.

Two bout exercise protocol (TBEP)

Upon arrival at the laboratory, the subjects relaxed before the first blood sample was drawn (Rest 1). Then the morning maximal incremental cycling ergometer test to exhaustion was performed, followed by the second blood sample (Ex 1). Lunch was provided (ad libitum), and the subjects rested during the remainder of the time between the exercise bouts. The afternoon exercise bout started 4 hours after the beginning of the morning test. Again, blood samples were drawn directly before (Rest 2) and after (Ex 2) the test (Figure 3.1). Hormonal levels were expressed as absolute values (Rest 1, Ex 1, Rest 2, Ex 2) and in terms of percentage (Ex 1 as percentage of Rest 1, and Ex 2 as percentage of Rest 2).

Each exercise bout started with cycling (Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands, or; Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) at 80 W for 3 minutes, followed by 30 W (women) or 40 W (men) increments every 3 minutes. The test ended when the subject was unable to maintain a cadence of at least 60 rpm, despite strong verbal encouragement. Saddle and handle bar height were recorded during the first test, and the same settings were used in all subsequent tests. Peak power output was defined as the average power output over the last 3 minutes of the exercise test (i.e. $PPO = W_{compl} + W_{incr} * (t/180)$, where W_{compl} is the last completed workload, W_{incr} is the increment in workload, and t is the number of seconds in the last workload).

Plasma ACTH

Peripheral blood was collected from the antecubital vein in ethylenediaminetetraacetic acid (EDTA) and heparin containing vacutainers. Blood collected in the EDTA tubes was centrifuged 1500 g for 10 min and the plasma fraction was frozen at -30°C for maximally 8 days (as part of a larger study protocol) and then stored at -80 °C until further analysis. Plasma ACTH concentrations were measured using an electrochemiluminescence

immunoassay on the Cobas E411 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The lower limit of detection was 2.0 ng·L^{-1} and inter-assay variation was 5.7 – 3.5% at 8.5 – 170 ng·L^{-1} respectively (N=18). Intra-assay variation was <1.2%. Normative values: morning measurements 5 - 70 ng·L^{-1}; afternoon measurements < 50 ng·L^{-1}.

Serum cortisol, growth hormone and prolactin

The heparinized samples were immediately cooled in a refrigerator at 4 °C for at least 30 minutes and then spun in a refrigerated centrifuge at 1500 g for 10 minutes. Within 1 hour after the blood was drawn the samples were stored at -30 °C for maximally 8 days (as part of a larger study) and then stored at -80 °C until further analyses. Serum concentrations of cortisol and PRL were determined by a chemiluminescence immunoassay on a Unicel Dxl 800 (Beckman Coulter, Bea, CA, USA). Inter-assay variation for cortisol was 5.4 – 8.2 % at 181 – 381 μg·L^{-1}, respectively. Intra-assay variation was 3.4 – 6.2 % at 181 – 381 μg·L^{-1}, respectively. Normative values: morning measurements 72.5-235.6 μg·L^{-1}; afternoon measurements 36.2-126.9 μg·L^{-1}. The lower limit of detection for PRL was 0.005 IU·L^{-1} and inter-assay variation was 1.42 – 1.61 % at 0.09 – 0.50 IU·L^{-1}, respectively. Intra-assay variation 6.92 – 3.32 % at 0.09 – 0.50 IU·L^{-1}, respectively (N=60). Normative values: female 0.02-0.53 IU·L^{-1}; male 0.02-0.42 IU·L^{-1}.

GH was measured using an immunometric technique on an IMMULITE 1000 analyser (Siemens Medical Solutions Diagnostics, Los Angeles, USA). The lower limit of detection was 0.15 mIU·L^{-1} and inter-assay variation was 7.4 – 3.7% at 0.42 – 24 mIU·L^{-1} respectively (N=70). Intra-assay variation was <2.0% and the normative value: <20 mIU·L^{-1}.

Classification: AF or FOR

Underperformance was used as the single criterion to determine FOR, because

it is one of the main diagnostic characteristics of FOR (Meeusen et al. 2013). In accordance with previous research (Aubry et al. 2014; Le Meur et al. 2014; Decroix, Piacentini, et al. 2016; ten Haaf et al. 2017), subjects were classified as FOR if the pre versus post TFL decrease in performance was bigger than the smallest worthwhile change in both the morning and afternoon exercise tests. The smallest worthwhile change was calculated as 0.3 times the coefficient of variation of peak power output, calculated between the pre TFL morning and afternoon exercise test (Hopkins, Hawley, and Burke 1999).

Statistical analyses

The Shapiro-Wilks test revealed that the hormonal data were not normally distributed. These data were log-transformed for further analyses, and displayed in text and figures as median and interquartile range. Linear mixed models were used to analyse differences between visits for physical performance, absolute hormonal levels, and responses in terms of percentage to the morning and afternoon exercise bout. Visit (pre, post, follow-up), classification (AF, FOR) and the visit by classification interaction were included in the model. Sex and timeslot (0815h, 0930h, 1045h) were included as covariates to control for sex differences and circadian rhythms of hormones. Significant effects were followed by pairwise comparisons. P-values smaller than .05 were considered statistically significant. Analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0, Amonk, NY).

3.3 Results

Subjects

One subject was excluded from analyses due to asthmatic symptoms during the post TFL exercise tests. The other subjects were classified as AF (N=14) or FOR (N=15), based on the criterion of the smallest worthwhile change

Table 3.1. Subject characteristics (mean±SD).

	AF (N=14)	FOR (N=15)	P-value
Sex (m/f)	11/3	7/8	.13
Age (yrs)	42.6 ± 11.0	39.1 ± 10.7	.39
Body mass index ($kg \cdot m^{-2}$)	24.0 ± 1.7	23.0 ± 2.3	.22
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.3 ± 6.2	51.8 ± 6.6	.83
Peak power ($W \cdot kg^{-1}$)	4.15 ± 0.61	4.06 ± 0.52	.64

AF: acute fatigued, FOR: functional overreached

in pre versus post TFL performance (coefficient of variation=1.6%, smallest worthwhile change= 0.5%). AF and FOR did not differ significantly in sex, age and baseline fitness level (Table 3.1). Four subjects dropped out at follow-up due to injuries. Some blood samples at pre TFL, post TFL and follow-up were missing due to technical errors. The numbers of subjects included in the analyses are indicated in Figures 3.3-3.6.

Performance (peak power output) in FOR, but not in AF, was significantly lower after the TFL than pre TFL (Figure 3.2). This was a consequence of our classification criterion. Post TFL performance in FOR was 0.23 $W \cdot kg^{-1}$ (95% confidence interval [0.08, 0.37]) lower in the morning exercise test, and 0.20 $W \cdot kg^{-1}$ [0.05, 0.35] in the afternoon test. At follow-up, performance was 0.19 $W \cdot kg^{-1}$ [0.05, 0.32] and 0.15 $W \cdot kg^{-1}$ [0.01, 0.30] lower than pre TFL in the morning and afternoon exercise test, respectively.

Resting hormonal levels

Figures 3.3A-3.6A show the resting hormonal levels (Rest 1) for ACTH, cortisol, GH and PRL, respectively. ACTH was significantly higher pre compared to post TFL (pre TFL: median 30 $\mu g \cdot L^{-1}$, interquartile range [18, 40], post TFL: 22 $\mu g \cdot L^{-1}$ [17, 28], P<.01). GH pre TFL (3.45 $mIU \cdot L^{-1}$ [0.54, 19.50]) was higher than post TFL (0.53 $mIU \cdot L^{-1}$ [0.18, 4.80], P<.001) and follow-up (2.2 $mIU \cdot L^{-1}$ [0.27, 8.00], P=.04). GH post TFL was lower than

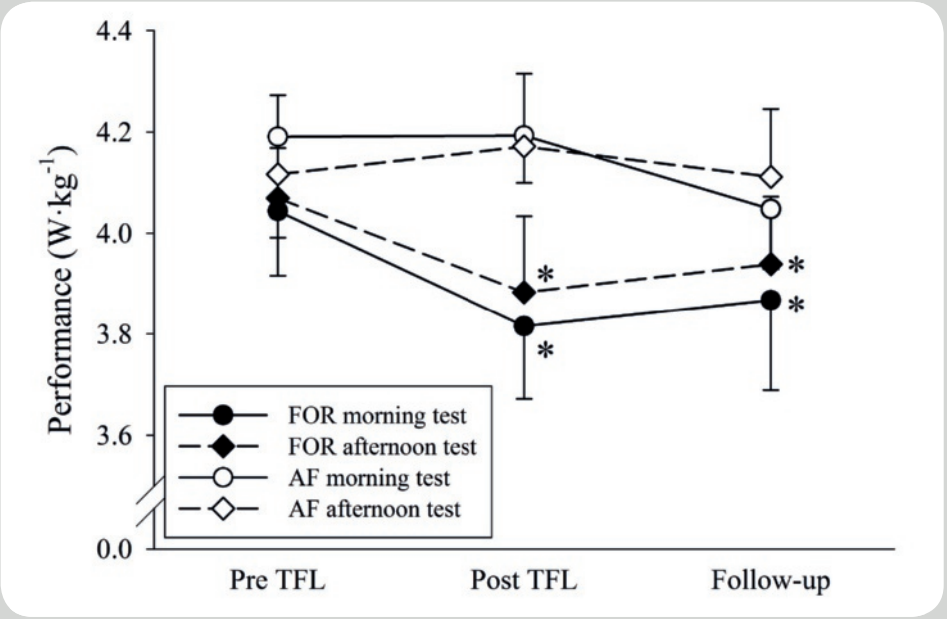


Figure 3.2. Physical performance (peak power output, W·kg^{-1}), in the morning (circles) and afternoon (diamonds) exercise tests for acute fatigued (open symbols) and functional overreached (filled symbols) athletes. Data are displayed as mean and SD adjusted for repeated measures (Cousineau 2005). * different from pre TFL (P<.05).

follow-up (P=.048). No effect of visit was observed for cortisol (F(2,50.7)=0.72, P=.49) and PRL (F(2,49.3)=0.07, P=.93).

Hormonal response to exercise

The exercise-induced hormonal responses were expressed as absolute levels and in terms of percentage (Ex 1 and Ex2 in Figures 3.3-3.6 A and D, respectively). The ACTH response to the morning exercise test (Ex 1) was significantly higher pre TFL (207 μg·L^{-1} [121, 268]) than post TFL (126 μg·L^{-1} [80, 220], P=.02) and follow-up (123 μg·L^{-1} [88, 264], P=.043), but not in terms of percentage (F(2,45.4)=0.38, P=.69). Also cortisol and PRL responses were higher pre than post TFL and at follow-up, both in absolute

values (F(2,48.2)=11.7, P<.001, and F(2,47.1)=7.0, P<.01, respectively), and in terms of percentage (F(2,49.4)=3.8, P=.03, and F(2,47.3)=5.4, P<.01, respectively). The GH response to the morning exercise test was lower pre (1019 % [306, 7768]) than post TFL (3172 % [361, 16972], P<.01) when expressed in terms of percentage, but not as absolute values (F(2,47.9)=2.2, P=.12).

In contrast to the response to the morning exercise test, no differences were observed in response to the afternoon exercise test (Ex 2), except for cortisol. The absolute cortisol response to the afternoon test pre TFL (120 $\mu g \cdot L^{-1}$ [94, 159]) was significant higher than post TFL (116 $\mu g \cdot L^{-1}$ [91, 127], P<.001,). In terms of percentage, post TFL (125 % [100, 144]) was lower than pre TFL (139 % [112, 175], P=.01) and follow-up (155 % [124, 194], P<.01).

Acute fatigue versus functional overreaching

None of the observed effects in resting hormonal levels (Rest 1) or responses to exercise (Ex 1, Ex 2) were different between AF and FOR, as the visit by classification interaction terms were not significant (Figures 3.3-3.6 B-C and E-F). The only main effect of classification was found for the ACTH response in terms of percentage to the morning exercise test (F(1,21.8)=4.5, P=.045). This indicated that the ACTH response was higher in AF than FOR, regardless of the visit.

3.4 Discussion

In this study it was investigated whether ACTH, cortisol, GH and PRL changed after an 8-day non-competitive amateur cycling event, and whether hormonal changes were related to the change in performance. Our results 1) showed that resting levels of ACTH and GH decreased, and 2) confirmed the hypothesis that exercise-induced hormonal responses were reduced after the event, but 3) rejected the hypothesis that hormonal changes were different

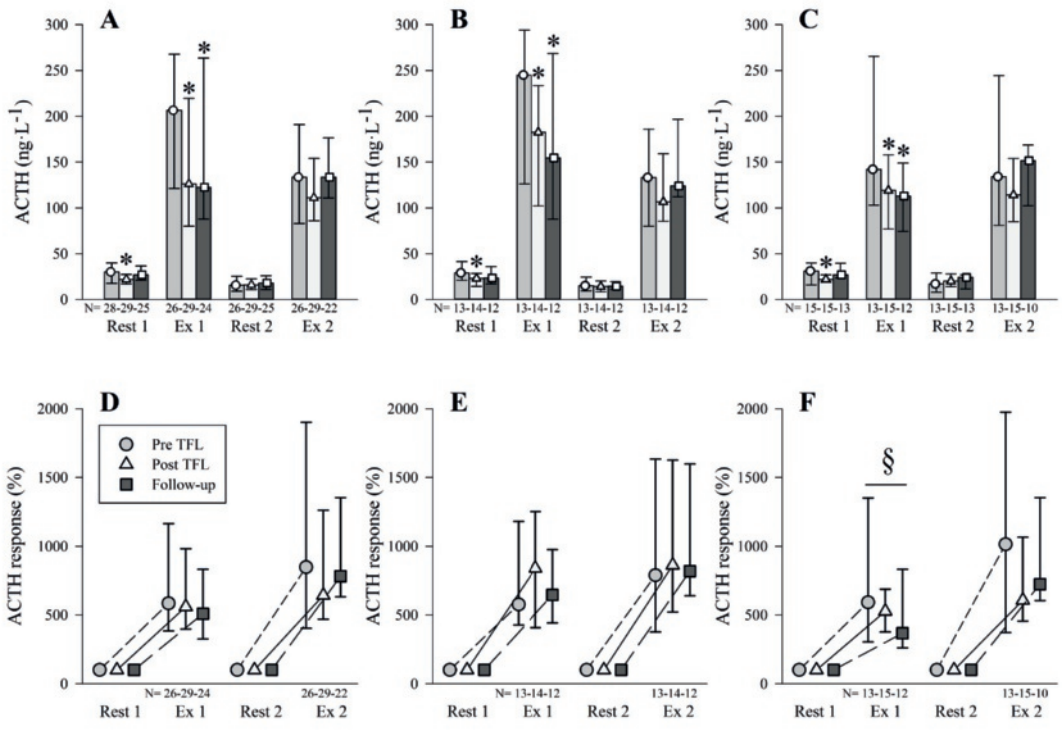


Figure 3.3. Absolute ACTH levels for all subjects together (**A**), acute fatigued (**B**), and functional overreached athletes (**C**), as well as exercise-induced responses in terms of percentage (Ex 1 relative to Rest 1, and Ex 2 relative to Rest 2) for the total group (**D**), acute fatigued (**E**) and functional overreached subjects (**F**). Median values are shown and error bars display the 25th and 75th percentiles. Numbers below the x-axis indicate the number of subjects included in analyses. * Significant different from pre TFL (P<.05); § Significant different from acute fatigued subjects (P<.05).

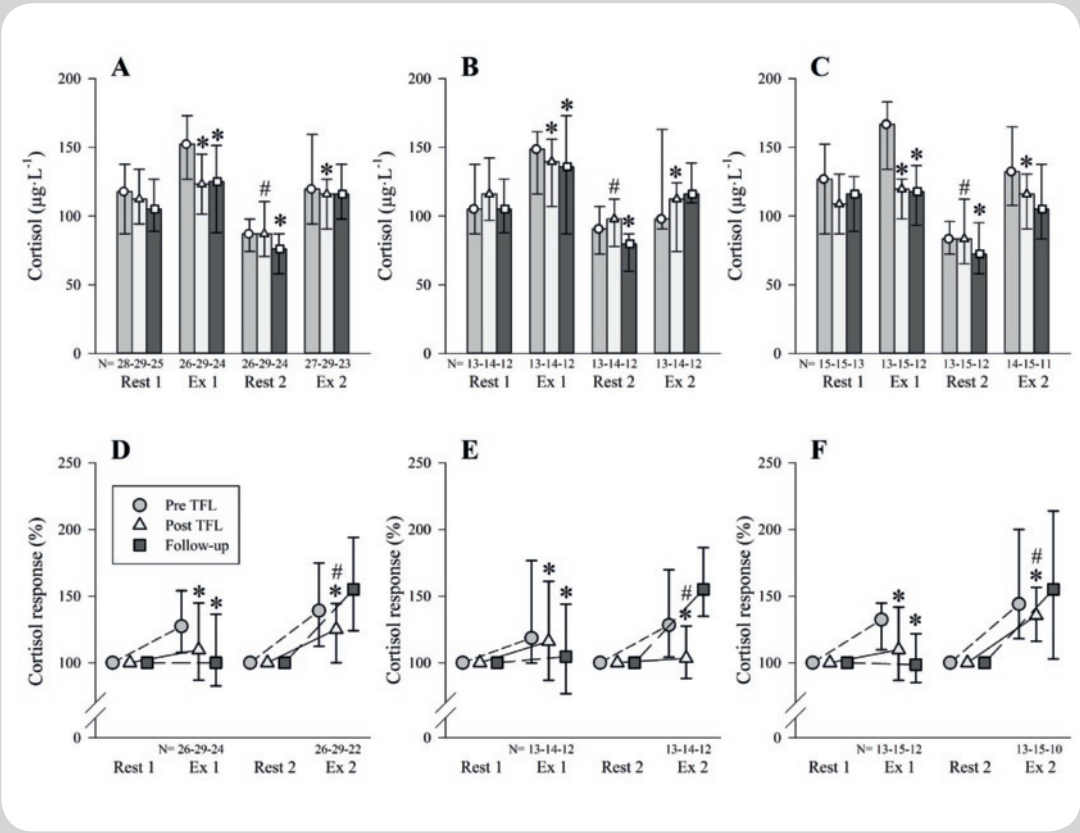


Figure 3.4. Absolute cortisol levels for all subjects together (**A**), acute fatigued (**B**), and functional overreached athletes (**C**), as well as exercise-induced responses in terms of percentage (Ex 1 relative to Rest 1, and Ex 2 relative to Rest 2) for the total group (**D**), acute fatigued (**E**) and functional overreached subjects (**F**). Median values are shown and error bars display the 25th and 75th percentiles. Numbers below the x-axis indicate the number of subjects included in analyses. * Significant different from pre TFL (P<.05); # Significant different from follow-up (P<.05).

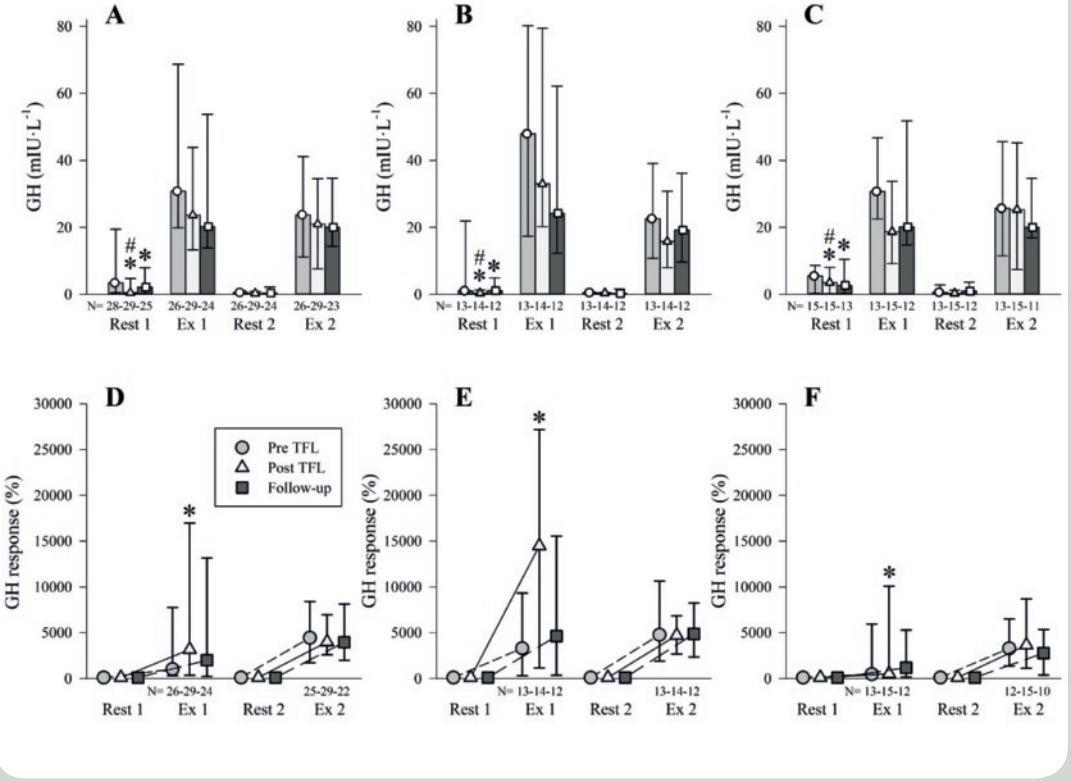


Figure 3.5. Absolute growth hormone levels for all subjects together (**A**), acute fatigued (**B**), and functional overreached athletes (**C**), as well as exercise-induced responses in terms of percentage (Ex 1 relative to Rest 1, and Ex 2 relative to Rest 2) for the total group (**D**), acute fatigued (**E**) and functional overreached subjects (**F**). Median values are shown and error bars display the 25th and 75th percentiles. Numbers below the x-axis indicate the number of subjects included in analyses. * Significant different from pre TFL (P<.05); # Significant different from follow-up (P<.05).

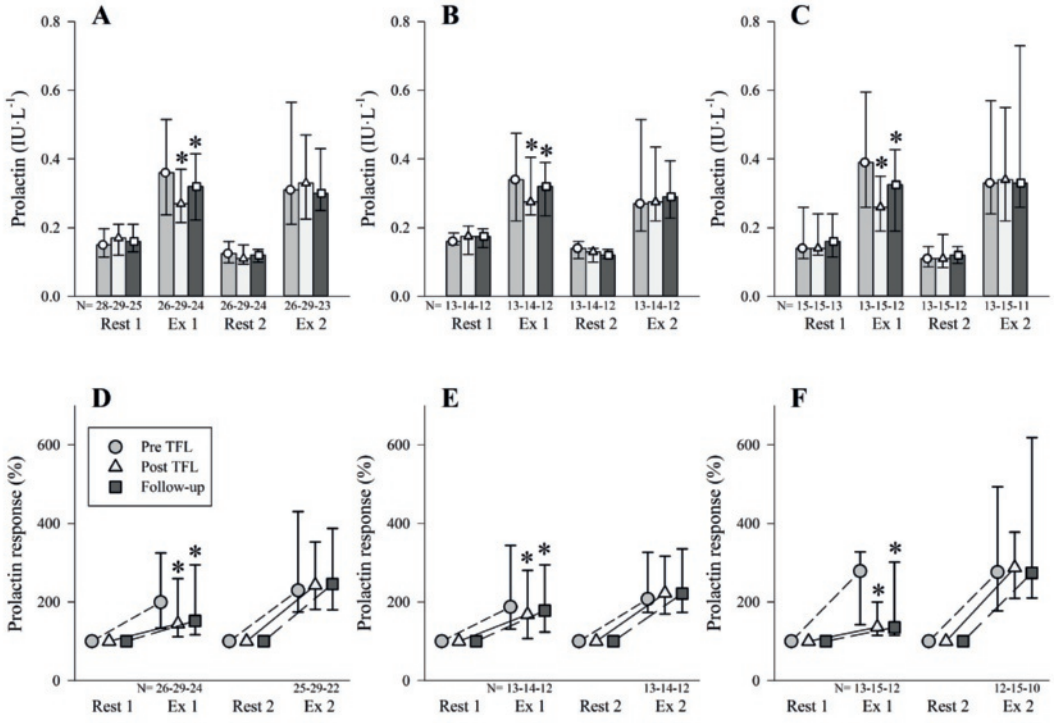


Figure 3.6. Absolute prolactin levels for all subjects together (**A**), acute fatigued (**B**), and functional overreached athletes (**C**), as well as exercise-induced responses in terms of percentage (Ex 1 relative to Rest 1, and Ex 2 relative to Rest 2) for the total group (**D**), acute fatigued (**E**) and functional overreached subjects (**F**). Median values are shown and error bars display the 25th and 75th percentiles. Numbers below the x-axis indicate the number of subjects included in analyses. * Significant different from pre TFL (P<.05).

between AF and FOR athletes.

Resting hormonal levels

In contrast to our first hypothesis, resting ACTH and GH were lower post than pre TFL. Most other studies reported no change in resting GH (Hecksteden et al. 2016; Lehmann et al. 1992; Meeusen et al. 2004; Rietjens et al. 2005), although recent research revealed lower resting GH in OTS compared to healthy athletes (Cadegiani and Kater 2017a). Literature on ACTH after intensified endurance training is ambiguous. Some studies confirmed our results and observed decreased resting ACTH (Hecksteden et al. 2016; Svendsen et al. 2016; Urhausen, Gabriel, and Kindermann 1998), while others found no change (Coutts, Wallace, and Slattery 2007; Meeusen et al. 2004; Rietjens et al. 2005). Interestingly, resting levels of the effector hormone of ACTH, cortisol, remained unchanged throughout our study. Unaltered resting cortisol levels after intensified endurance training have been reported frequently in the literature (Coutts, Wallace, and Slattery 2007; Hecksteden et al. 2016; Meeusen et al. 2004; Rietjens et al. 2005; Steinacker et al. 2000). It is unlikely that the apparent dissociation between resting ACTH and cortisol in our study resulted from (mal)adaptations of the adrenals, since a previous study showed a normal cortisol response to pharmacological adrenal stimulation (Cadegiani and Kater 2017c). Therefore, an increased adrenal mass, for example observed in long-term exercising rodents, or modulation of the adrenal sensitivity are improbable (Bornstein et al. 2008). However, the ACTH-cortisol relation might be modulated by ACTH-independent stimulation (e.g. by interleukine-6) or an altered clearance rate of cortisol (Bornstein et al. 2008). These modulating mechanisms are affected by several factors that are possibly altered after intensified training, such as catecholamines and immune factors (Lehmann et al. 1991; Svendsen et al. 2016).

Hormonal response to exercise

The results of our study confirmed the hypothesis that exercise-induced hormonal responses were reduced after the TFL. Reduced hormonal responses were demonstrated previously in the most severe stage of the overtraining spectrum (OTS) (Barron et al. 1985; Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). We showed that lower ACTH, cortisol and PRL responses to exercise can also be observed earlier in the overtraining spectrum (i.e. AF and FOR).

GH response to the morning exercise bout in terms of percentage was increased. This, however, seemed the result of a lower resting level, rather than an increased response to exercise (Rest 1 versus Ex 1, Figure 3.5A). A decreased cortisol response after intensified training was shown in most studies (Lehmann et al. 1992; Meeusen et al. 2004; Rietjens et al. 2005; Uusitalo et al. 1998), although some studies observed no change (Svendsen et al. 2016; Urhausen, Gabriel, and Kindermann 1998). Only two studies presented PRL responses after intensified training, and both did not observed a change (Lehmann et al. 1992; Meeusen et al. 2004). Equivocal results have been reported for ACTH, with some studies confirming our result (Meeusen et al. 2004; Urhausen, Gabriel, and Kindermann 1998) while others found no effect (Rietjens et al. 2005; Svendsen et al. 2016). The inconsistent results in the literature may be a consequence of the small sample size in the previous studies (N=7-15), various training status (no change in performance, decreased performance, OTS diagnosed by physicians), and/or different training protocols (8 days to 6 weeks, 30% to 150% volume increase). As pointed out previously in a recent review of the literature, this lack of standardization in the limited number of available studies makes comparison of results difficult (Cadegiani and Kater 2017b).

Our and previous results (Meeusen et al. 2004; Urhausen, Gabriel, and Kindermann 1998) do not support the biphasic response theory (Lehmann et al. 1998), that suggests a gradual increase in ACTH response in overreached athletes, followed by a decreased response in the most advanced stage of

the overtraining spectrum (OTS). Instead, the current findings suggest that the hormonal adaptation acquired by training is apparently lost after the 8 day non-competitive cycling event, as suggested previously in OTS athletes (Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). This loss of hormonal adaptation post TFL is most likely a result of overreaching, rather than detraining, since cortisol and GH levels have been shown to be unchanged after 5 days of detraining (Mujika and Padilla 2000). It is unclear whether the loss of adaptation (decreased ACTH, cortisol and PRL responses) at follow-up is a result of overreaching or detraining. Some subjects achieved their goal by finishing the TFL and refrained from training after the TFL, while others continued their training. Barron et al. (1985) showed that hormonal disturbances recovered after 4 weeks of adequate rest. For that reason, the lack of recovery of hormonal responses at follow-up in our study was possibly due to detraining effects.

In contrast to our hypothesis, reduced hormonal responses to the morning, but not to the afternoon exercise test were observed. The only exception being the cortisol response, which was reduced for the morning and afternoon exercise test. In previous studies that applied the TBEP, the hormonal changes were most pronounced in the afternoon exercise bout (Meeusen et al. 2004; Meeusen et al. 2010). Meeusen et al. (2004) showed that the cortisol response in terms of percentage was reduced after a training camp, which mainly resulted from a higher resting level (Rest 2). It was, therefore, suggested that the recovery capacity was reduced after intensified training. This reduced recovery capacity was not confirmed by our results, as the afternoon resting (Rest 2) levels were not different before and after the TFL.

Acute fatigue versus functional overreaching

Activation of the pituitary and its associated hormones ACTH, cortisol, GH and PRL represent a physiological response to the energetic and metabolic needs of exercise (Duclos, Guinot, and Le Bouc 2007). For that reason, it was

hypothesized that cyclists who underperformed after the TFL (FOR) would show decreased hormonal responses, whereas changes would be absent or less noticeable in subjects who maintained their performance level (AF). Yet, no visit by classification interaction effects were found in our study. This means that the observed reduced hormonal responses were not different between AF and FOR. Subjects in our study were classified as either AF or FOR based on the smallest worthwhile change in performance. This classification criterion remains an arbitrary choice and might affect the results. Yet, the criterion in our study is based on the most important diagnostic characteristic of overreaching (i.e. underperformance), and is in line with several previous studies (Aubry et al. 2014; Decroix, Piacentini, et al. 2016; ten Haaf et al. 2017). To the best of our knowledge, the current study is the first that compared longitudinal changes in exercise-induced ACTH, cortisol, GH and PRL responses between athletes whose performance level was maintained (AF) or decreased (FOR) after imbalanced exercise and recovery. Nevertheless, reduced hormonal responses have been shown in studies in which subjects underperformed (Lehmann et al. 1992; Uusitalo et al. 1998) as well as in studies in which subjects maintained their performance level after intensified training (Meeusen et al. 2004; Rietjens et al. 2005). This led us to suggest that reduced exercise-induced ACTH, cortisol and PRL responses are a general effect of the 8-day non-competitive amateur cycling event, rather than the performance limiting factor. Apparently, other physiological or psychological factors coincided with the altered hormonal levels that (jointly) determined the change in performance.

Limitations

The relative increase in training load applied in our study (9 fold) is larger than expected in elite athletes. Caution must, therefore, be taken when extrapolating these results to training practice in elite athletes. Instead, this research improves understanding of hormonal disturbances that occur in

the overtraining spectrum, and adds to the knowledge on diagnosing an undesirable training status.

3.5 Conclusion

The results of this study support the hypothesis that exercise-induced ACTH, cortisol and PRL responses were reduced after an 8-day non-competitive amateur cycling event. This illustrates that hormonal adaptations can occur already in the early stage of the overtraining spectrum (i.e. AF and FOR). The observed hormonal changes were not related to underperformance, but seemed rather a general result of the 8 day cycling event.

Chapter 4

Heart rate seems inadequate to prescribe and monitor intensified training

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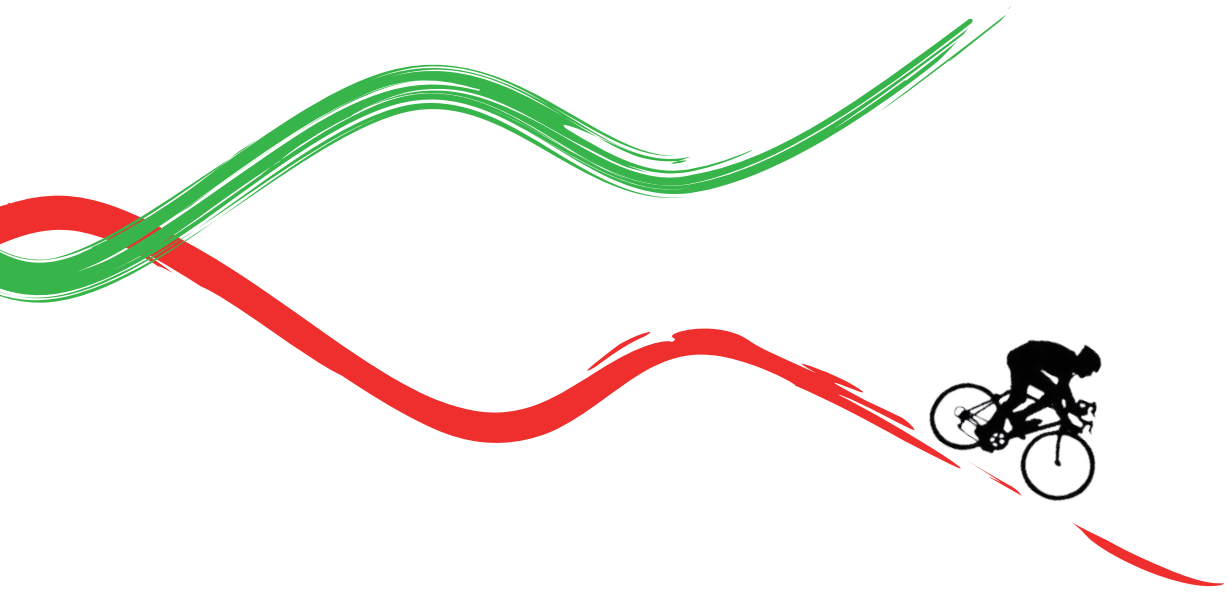
Submitted for publication



Abstract

The aim of this study was to investigate whether the change in (sub) maximal heart rate after intensified training is associated with the change in performance. Thirty subjects were recruited who performed cardiopulmonary exercise tests to exhaustion 2 weeks before (pre), 1 week after (post) and 5 weeks after (follow-up) an 8-day non-competitive amateur cycling event (TFL). The exercise volume during the TFL was 9 fold the volume during the preparation period. Heart rate and cardiopulmonary parameters were obtained at standardized absolute submaximal workloads (low, medium and high intensity) and at peak level each test. Subjects were classified as functional overreached (FOR) or acute fatigued (AF) based on the change in performance. No differences between FOR and AF were observed for heart rate (P=.51). On total group level (AF+FOR), post TFL heart rate decreased significantly at low (-4.4 beats·min^{-1}, 95% CI [-8.7, -0.1]) and medium (-5.5 beats·min^{-1} [-8.5, -2.4]), but not at high intensity. Peak heart rate decreased -3.4 beats·min^{-1} [-6.1, -0.7]. O_2pulse was on average 0.49 ml O_2·beat^{-1} [0.09, 0.89] higher at all intensities after intensified training. No changes in V̇O2 (P=.44) or the ventilator threshold (P=.21) were observed. Pearson's correlation coefficients revealed negative associations between heart rate and O_2pulse at low (r=-.56, P<.01) and medium intensity (r=-.54, P<.01), but not with $\dot{V}O_2$ or any other submaximal parameter. In conclusion, (sub) maximal heart rate decreased after the TFL. However, this decrease is unrelated to the change in performance. Therefore, heart rate seems inadequate to prescribe and monitoring intensified training.

4.1 Introduction

Overtraining is a process of intensified training with insufficient rest, resulting in accumulated fatigue and decreased performance. The possible expression of overtraining is functional overreaching (FOR), non-functional overreaching (NFOR) or eventually overtraining syndrome (OTS) (Meeusen et al. 2006; Meeusen et al. 2013). FOR is sometimes deliberately induced in training programs, whereas NFOR and OTS should be avoided because of the severity of symptoms and the long or even absent recovery times. It is, therefore, important to appropriately prescribe exercise and monitor training status.

Heart rate can easily be measured and is often used to prescribe and monitor training (Achten and Jeukendrup 2003). Maximal heart rate is generally reduced after an intensified training period (Meeusen et al. 2013), but the effect on submaximal heart rate is not completely clear. Bosquet and colleagues (2008) concluded in their review that submaximal heart rate was lower after periods of intensified training of more than 2 weeks, but unchanged after periods shorter than 2 weeks. Yet, a lower submaximal heart rate has also been shown after an 8-day training camp (Decroix, Lamberts, and Meeusen 2018). Furthermore, it is unknown whether the possible change in heart rate is associated with underperformance (i.e. FOR) after an intensified training period. For example, some studies have used elevated submaximal heart rate as an indicator of overreaching (Brink, Visscher, Coutts, et al. 2012; Schmikli et al. 2012). In contrast, Le Meur et al. (2014) showed that (sub) maximal heart rate was lower after intensified training, regardless whether athletes underperformed (FOR) or not after this period. Interestingly, only athletes who underperformed (FOR) showed a decrease in cardiac output, resulting from a decreased stroke volume rather than a drop in heart rate.

The aim of this study was to investigate the effect of intensified training on (sub) maximal heart rate. A second goal was to examine whether the potential change in (sub) maximal heart rate was associated with a change in physical performance. For this purpose, we evaluated cardiopulmonary exercise tests before and after an 8-day non-competitive amateur cycling event that

possibly resulted in underperformance (FOR). Subjects who underperformed as a result of the event (FOR) were compared with subjects who maintained their performance level, and correlation analyses were performed. It was hypothesized that (sub) maximal heart rate would be lower after this period of intensified training, but that this decrease would not be associated with a change in performance.

4.2 Methods

Subjects

A total of 30 subjects were recruited (11 female, 19 male). $\dot{V}O_2$max at baseline (mean±SD) was 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$, peak power output was 4.12±0.57 $W \cdot kg^{-1}$ and age was 40.8±10.8 y. The subjects' fitness level can be described as performance level 1 (4%), 2 (57%), 3 (25%) and 4 (14%) according to the $\dot{V}O_2$max-based athlete classification norms (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016). Subjects gave written informed consent prior to the first measurements. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical committee (VUmc 2014.319).

Design

The subjects visited our laboratory 12-17 days before (pre), 5-8 days after (post) and 33-36 days after (follow-up) the Tour for Life (TFL). The TFL is an amateur non-competitive cycling event that covers a total of 1300 km and 18,500 vertical climbing meters in 8 consecutive days. A previous study (ten Haaf et al. 2017) showed that subjects cycled on average 495±66 minutes per day with a rating of perceived exertion (Foster et al. 1995) of 6.9±1.2 on a 0-10 visual analogue scale. The exercise volume was approximately 9 fold the exercise volume during the preparation period. During each laboratory

visit cardiopulmonary parameters and performance were evaluated by means of a maximal incremental cycling test to exhaustion. As part of a larger research project, subjects performed two tests during each visit, with 4 hours between the start of the morning and afternoon exercise test. Subjects did not perform vigorous exercise within 36 hours prior to the laboratory visits. Habitual breakfast was consumed at home, at the latest 2 hours before the each laboratory visit. Between the exercise bouts the subjects relaxed and lunch was provided.

Exercise tests

Each exercise bout started with cycling (Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands, or; Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) at 80 W for 3 minutes, increased by 30 W (women) or 40 W (men) every 3 minutes. The test ended when the subject was unable to maintain a cadence of at least 60 rpm, despite strong verbal encouragement. Each subject performed the tests on the same ergometer and at the same time of the day during each laboratory visit. Saddle and handle bar height were recorded after the first test, and the same settings were used in all subsequent tests. Performance was defined as the peak power output (PPO) achieved each test. PPO was calculated as the average power output over the last 3 minutes of the exercise test (i.e. $PPO = W_{compl} + W_{incr} * (t/180)$, where W_{compl} is the last completed workload, W_{incr} is the increment in workload, and t is the number of seconds in the last workload).

Cardiopulmonary measurements

Heart rate was measured beat-by-beat using a portable electrocardiography device (VU-AMS; Vrije Universiteit Amsterdam, the Netherlands). Both 5 and 7 lead versions of the VU-AMS5fs device (De Geus et al. 1995) together with Kendal ARBO H98SG single use electrodes were used. Gas exchange

was measured breath-by-breath using open circuit spirometry (Cosmed Quark CPET; and Cosmed Quark B2, Cosmed S. R. L., Rome, Italy). The gas analysers and volume transducers were calibrated before every test (gas analysers: reference gas mixture of 16.00% O_2 and 5.00% CO_2, volume transducers: 3 litre syringe). The same machine was used for each individual during each visit.

Data analyses

A moving median filter was applied on the breath-by-breath respiratory data and the beat-by-beat heart rate data to remove outliers. The ventilatory threshold (L $O_2 \cdot min^{-1}$) was determined by two blinded assessors according to the v-slope method (Beaver, Wasserman, and Whipp 1986). Thresholds that differed more than 2% between assessors were discussed until consensus was reached.

For further analyses data were converted into second-by-second data using interpolation. Heart rate (beats$\cdot min^{-1}$), O_2pulse (ml $O_2 \cdot beat^{-1}$), $\dot{V}O_2$ (L $O_2 \cdot min^{-1}$), $\dot{V}CO_2$ (L $CO_2 \cdot min^{-1}$), respiratory exchange ratio (RER) and pulmonary ventilation (L$\cdot min^{-1}$) were obtained at standardized absolute submaximal workload (low, medium and high intensity) and at peak levels. Low exercise intensity was defined as the first exercise step (80 W). Medium and high intensity were defined as 50% PPO and 100% PPO of the worst performance of the three visits, respectively. Worst performance was determined per subject as the lowest PPO of the 6 tests, so that the cardiopulmonary parameters were calculated at the same absolute power output for each exercise test. 50% of worst performance was rounded to the lower exercise step. For example, a male subject whose lowest peak power output in the 6 tests was 328 W completed at least 7 exercise steps every test (i.e. 80-120-160-200-240-280-320 W). In this example, the parameters for all 6 exercise tests were calculated over the last 30 seconds of the first step (80 W), third step (160 W, 50% worst performance), seventh step (320 W, 100%

worst performance) and the highest 30 second average (peak). All outcome parameters were averaged over the morning and afternoon exercise test for each visit.

Classification: AF or FOR

Subjects were classified as either functional overreached (FOR) or acute fatigued (AF) based on the change in physical performance, since underperformance is one of the main characteristics of FOR (Meeusen et al. 2013). In line with previous research, subjects were classified as FOR if the decrease in performance between the pre and post TFL exercise tests was bigger than the smallest worthwhile change (Aubry et al. 2014; Decroix, Piacentini, et al. 2016; ten Haaf et al. 2017). The smallest worthwhile change was calculated as 0.3 * the coefficient of variation of peak power output between the 2 pre TFL exercise tests (Hopkins, Hawley, and Burke 1999).

Statistical analyses

Normality of the data was confirmed after checking using the Shapiro-Wilks test. Repeated-Measures ANOVAs with visit (pre, post and follow-up) and intensity (low, medium, high and peak intensity) as within-subject factors and classification (FOR, AF) as between-subject factor were used to analyse differences in heart rate, O_2pulse, $\dot{V}O_2$, $\dot{V}CO_2$, RER and pulmonary ventilation. Differences between visits for the ventilatory threshold and physical performance (PPO) were analysed using Repeated-Measures ANOVAs with visit (pre, post and follow-up) as within-subject factor and classification (FOR, AF) as between-subject factor. Significant main effects were followed by Bonferroni adjusted pairwise comparisons. Pearson's correlation coefficients were calculated to investigate the associations between the pre versus post TFL changes of the cardiopulmonary measures at each exercise intensity (heart rate, O_2pulse, $\dot{V}O_2$, $\dot{V}CO_2$, RER and pulmonary ventilation),

Table 4.1. Subject characteristics (mean±SD).

	AF (N=12)	FOR (N=12)	P-value
Sex (m/f)	10/2	7/5	.18
Age (yrs)	44.0 ± 10.8	38.9 ± 11.2	.27
Body mass index ($kg \cdot m^{-2}$)	24.1 ± 1.8	23.1 ± 2.5	.27
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.3 ± 5.6	52.1 ± 6.8	.80
Peak power ($W \cdot kg^{-1}$)	4.22 ± 0.53	4.12 ± 0.55	.63

AF: acute fatigued, FOR: functional overreached

the ventilatory threshold and physical performance. The standard deviations shown in figures were adjusted for repeated measures by means of excluding between-subject variability (Cousineau 2005). P-values smaller than .05 were considered statistically significant. Analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0, Amonk, NY).

4.3 Results

Subjects

One subject was excluded from analyses because he showed asthmatic symptoms during to post TFL exercise tests. Three subjects dropped-out during the follow-up measurements due to injuries. Data of 3 subjects were missing due to technical errors. As a result, 24 subjects were included in the ANOVAs, and 26 in the correlation analyses.

The coefficient of variation of PPO in the pre TFL exercise tests was 1.6%, meaning that the smallest worthwhile change was 0.5%. According to the classification criteria, 12 subjects were categorised as FOR and 12 as AF. The baseline characteristics of these groups are displayed in Table 4.1. As a consequence of our classification criteria, PPO in FOR was 0.21 $W \cdot kg^{-1}$ (95% confidence interval [-0.38, -0.06]) lower post compared to pre TFL, but did

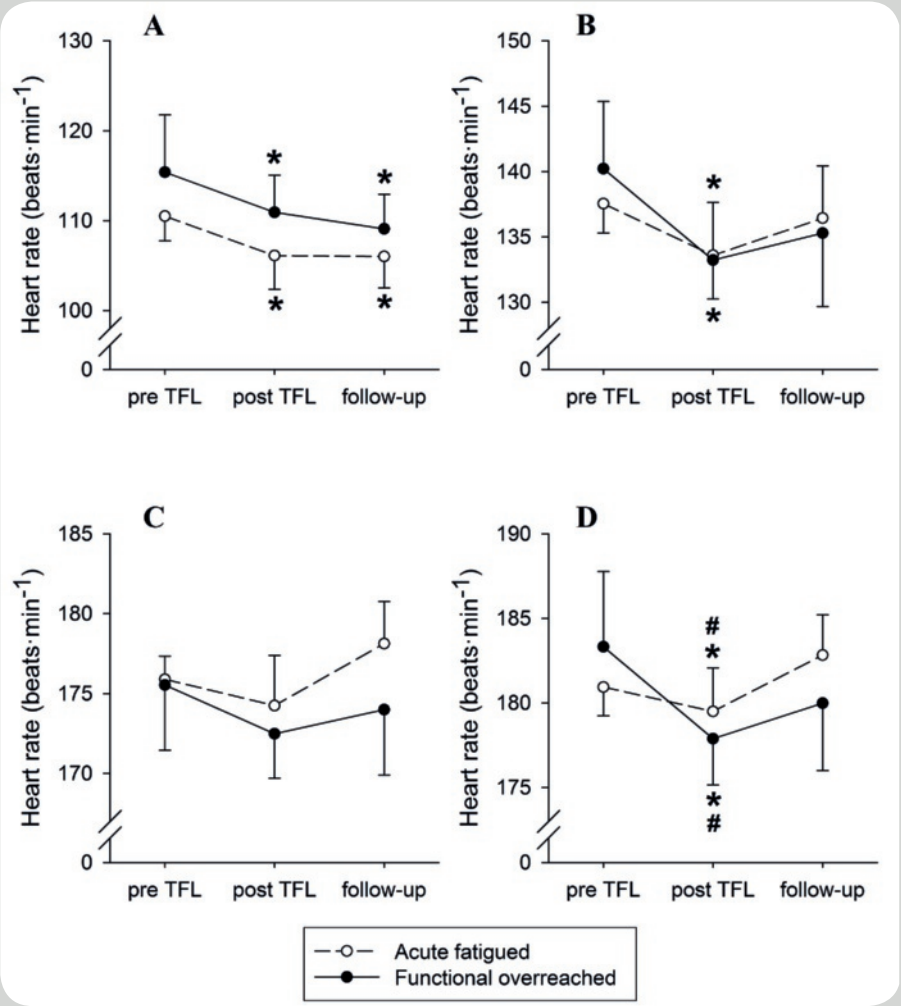


Figure 4.1. Heart rate at low (**A**, 80 W), medium (**B**, 50% worst performance), high (**C**, 100% worst performance) and peak (**D**) exercise intensity for acute fatigued (N=12, open circles) and functionally overreached subjects (N=12, filled circles). Data are displayed as mean ± adjusted SD (Cousineau 2005). * Significant different from baseline (P<.05). # Significant different from follow-up (P<.05).

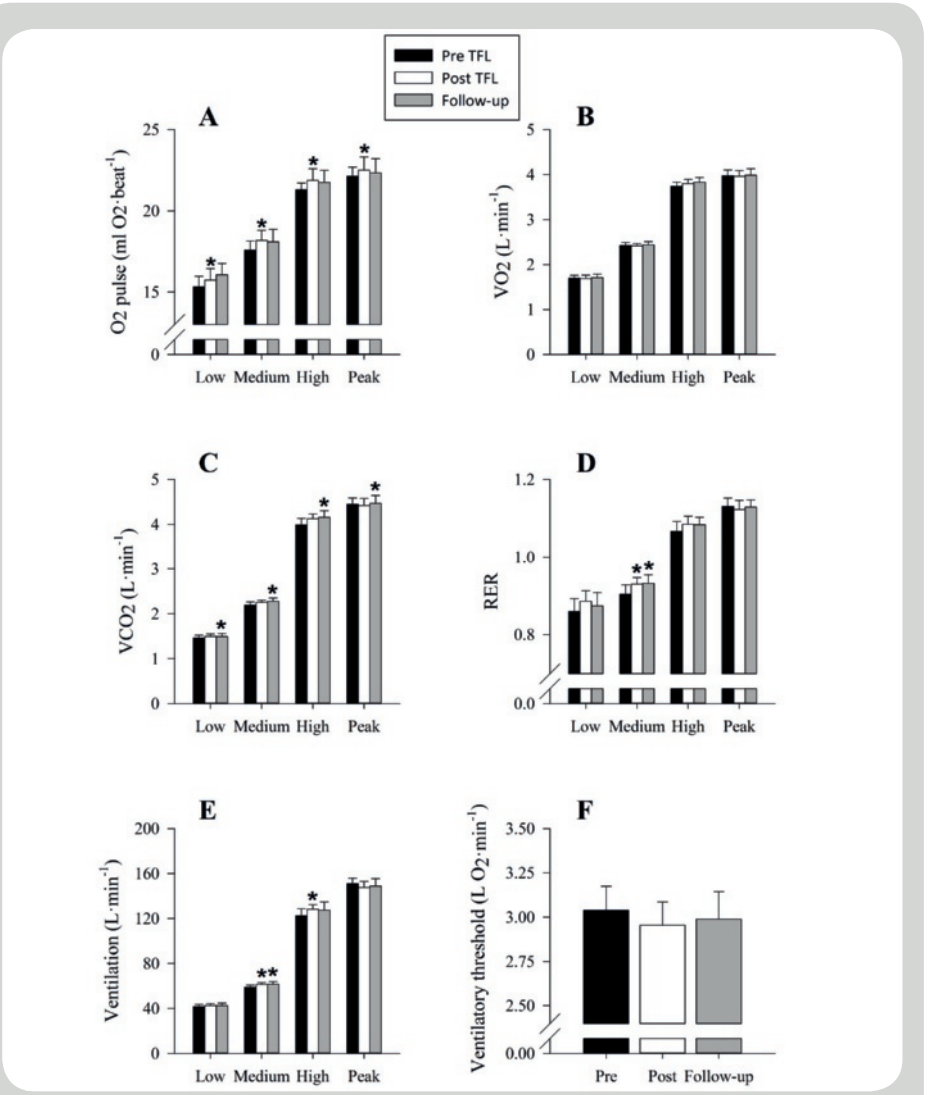


Figure 4.2. O2 pulse (**A**), $\dot{V}O_2$ (**B**), $\dot{V}CO_2$ (**C**), respiratory exchange ratio (**D**), pulmonary ventilation (**E**) and ventilator threshold (**F**) for the pre (black), post (white) TFL and follow-up (grey) incremental cycling ergometer tests. Data (N=24) are shown for low (80 W), medium (50% worst performance) and high intensity (100% worst performance) and peak values. Worst performance was determined per subject as the lowest peak power output of all tests, so that the cardiopulmonary parameters were calculated at the same absolute power output for each exercise test. Data are displayed as mean ± adjusted SD (Cousineau 2005). * Significant different from baseline (P<.05).

not change significantly in AF (0.02 W·kg^{-1} [-0.09, 0.13]). Also at follow-up, PPO was significantly lower in FOR (-0.22 W·kg^{-1} [-0.41, -0.02]) but not in AF (-0.06 W·kg^{-1} [-0.22, 0.11]).

Heart rate

No significant visit by classification ($F(2,44)=1.33$, $P=.27$, $\eta_p^2=.06$) or visit by intensity by classification ($F(2.8,62.0)=0.76$, $P=.51$, $\eta_p^2=.03$) interaction effect was observed for heart rate (Figure 4.1). On total group level (AF+FOR), the visit by intensity interaction was significant ($F(2.8,62.0)=6.50$, $P<.001$, $\eta_p^2=.23$). At low intensity, the heart rate post TFL (-4.4 beats·min^{-1}, 95% confidence interval: [-8.7, -0.1]) and at follow-up (-5.4 beats·min^{-1} [-9.5, -1.3]) were lower than pre TFL. At medium exercise intensity the heart rate was lower post compared to pre TFL (-5.5 beats·min^{-1} [-8.5, -2.4]), but at high exercise intensity no significant differences were observed between the visits. The peak heart rate was lower post TFL than pre TFL (-3.4 beats·min^{-1} [-6.1, -0.7]) and follow-up (-2.7 beats·min^{-1} [-5.3, -0.1]).

(Cardio)pulmonary parameters

In line with heart rate, none of the other cardiopulmonary parameters showed a significant visit by classification (O_2pulse: $P=.77$, $\dot{V}O_2$: $P=.96$, $\dot{V}CO_2$: $P=.55$, RER: $P=.35$, pulmonary ventilation: $P=.74$, ventilatory threshold: $P=.86$) or visit by intensity by classification interaction effect (O_2pulse: $P=.40$, $\dot{V}O_2$: $P=.43$, $\dot{V}CO_2$: $P=.69$, RER: $P=.18$, pulmonary ventilation: $P=.43$). The data for AF and FOR per visit are provided in Table 4.2.

On total group level (AF+FOR), a significant effect of visit was observed for O_2pulse ($F(1.69,38.8)=3.72$, $P=.04$, $\eta_p^2=.14$), without different effects between the exercise intensities. The O_2pulse post TFL was on average 0.49 ml O_2·beat^{-1} [0.09, 0.89] higher than pre TFL, but not significant different at follow-up (Figure 4.2 A). $\dot{V}O2$ (Figure 4.2 B) did not change between the

Table 4.2. Cardiopulmonary parameters (mean ± normal SD) for acute fatigued (N=12) and functionally overreached (N=12) subjects pre and post TFL and at follow-up. Data are displayed for low (80 W), medium (50% worst performance) and high intensity (100% worst performance) and peak values. Worst performance was determined per subject as the lowest peak power output of all tests, so that the parameters were obtained at the same absolute workload for each exercise test.

	Pre TFL		Post TFL		Follow-up	
	AF	**FOR**	**AF**	**FOR**	**AF**	**FOR**
Heart rate						
low	110	115	106*	111*	106*	109*
	±15	±17	±14	±11	±13	±12
Medium	138	140	134*	133*	136	135
	±14	±14	±14	±14	±14	±17
High	176	176	174	172	178	174
	±11	±12	±11	±12	±13	±13
Peak	181	183	179*#	178*#	183	180
	±10	±10	±11	±11	±11	±12
O_2pulse						
low	15.9	14.7	16.2*	15.3*	16.5	15.7
	±2.4	±2.2	±2.1	±1.9	±2.6	±1.6
Medium	18.3	16.8	18.6*	17.8*	18.6	17.6
	±2.8	±2.5	±2.5	±2.5	±3.4	±2.0
High	22.0	20.6	22.6*	21.1*	22.4	21.1
	±3.0	±3.3	±3.1	±3.7	±3.5	±3.3
Peak	22.7	21.6	23.2*	21.8*	22.9	21.8
	±3.0	±3.9	±3.0	±4.0	±3.5	±3.5
$\dot{V}O_2$						
low	1.728	1.671	1.694	1.682	1.718	1.698
	±0.117	±0.152	±0.147	±0.169	±0.158	±0.186
Medium	2.496	2.350	2.463	2.370	2.503	2.375
	±0.310	±0.363	±0.330	±0.369	±0.327	±0.387
High	3.870	3.610	3.940	3.658	3.980	3.680
	±0.526	±0.618	±0.606	±0.726	±0.627	±0.694

Peak	4.053	3.905	4.085	3.840	4.098	3.875
	±0.560	±0.737	±0.596	±0.832	±0.635	±0.729
$\dot{V}CO_2$						
low	1.458	1.462	1.469	1.518	1.495*	1.480*
	±0.108	±0.157	±0.140	±0.150	±0.144	±0.137
Medium	2.246	2.136	2.289	2.206	2.340*	2.207*
	±0.306	±0.360	±0.324	±0.348	±0.313	±0.355
High	4.188	3.793	4.283	3.967	4.368*	3.950*
	±0.591	±0.680	±0.690	±0.828	±0.720	±0.806
Peak	4.550	4.345	4.568	4.279	4.617*	4.321*
	±0.652	±0.820	±0.725	±0.927	±0.743	±0.820
RER						
low	0.85	0.88	0.87	0.90	0.87	0.87
	±0.04	±0.05	±0.05	±0.04	±0.05	±0.05
Medium	0.90	0.91	0.93*	0.93*	0.93*	0.93*
	±0.03	±0.04	±0.04	±0.04	±0.03	±0.04
High	1.08	1.05	1.09	1.08	1.10	1.07
	±0.04	±0.05	±0.04	±0.05	±0.04	±0.04
Peak	1.13	1.13	1.12	1.12	1.14	1.12
	±0.04	±0.04	±0.04	±0.05	±0.03	±0.02
Ventilation						
low	41	42	42	43	42	43
	±4	±5	±4	±4	±5	±4
Medium	60	58	62*	61*	62*	61*
	±8	±9	±8	±9	±7	±9
High	129	116	132*	124*	131	124
	±23	±25	±21	±27	±19	±31
Peak	151	151	150	146	149	149
	±24	±32	±26	±32	±26	±33
VT	3.164	3.012	3.083	2.921	3.125	3.016
	±0.388	±0.419	±0.466	±0.498	±0.427	±0.512
Performance	4.22	4.12	4.25	3.90*	4.17	3.90*
	±0.53	±0.55	±0.49	±0.54	±0.50	±0.50

*Significant different from pre Tour for Life (P<.05); # Significant different from follow-up (P<.05).

visits (F(2,44)=0.81, P=.45, η_p^2=.04), while $\dot{V}CO_2$ was 0.075 L $CO_2 \cdot min^{-1}$ [0.000, 0.149] higher at follow-up compared to pre TFL (Figure 4.2 C). Only at medium exercise intensity the RER was higher post TFL (0.03 [0.01, 0.04]) and at follow-up (0.03 [0.01, 0.05]) compared to pre TFL (Figure 4.2 D). Pulmonary ventilation (Figure 4.2 E) was higher post than pre TFL, but only at medium (2.40 $L \cdot min^{-1}$ [1.00, 3.81]) and high exercise intensity (5.93 $L \cdot min^{-1}$ [2.01, 9.84]). The ventilatory threshold (Figure 4.2 F) was not different between visits (F(2,44)=1.63, P=.21, η_p^2=.07).

Cardiopulmonary parameters and performance

Table 4.3 shows the associations between the changes in cardiopulmonary and performance data, calculated as the differences between pre and post TFL (e.g. heart $rate_{post\text{-}pre}$ versus $O_2pulse_{post\text{-}pre}$). A negative association was observed between the change in heart rate and O_2pulse at low (*r*=-.56, P<.01) and medium (*r*=-.54, P<.01), but not at high (*r*=-.30, P=.13) and peak exercise intensity (*r*=-.04, P=.86). Yet, the change in submaximal heart rate was not related to changes in $\dot{V}O_2$ or changes in any other parameter. In contrast, O_2pulse was positively associated with $\dot{V}O_2$ at every exercise intensity, with the strongest associations at high (*r*=.82, P<.001) and peak intensity (*r*=.85, P<.001). Performance was weakly negatively associated with the change in $\dot{V}CO_2$ at low (*r*=-.40, P=.04) and medium exercise intensity (*r*=-.44, P=.03). At high intensity, performance was negatively associated with the change in RER (*r*=-.54, P<.01) and pulmonary ventilation (*r*=-.47, P=.02). The peak values of all parameters changed positively with the change in performance, except RER (*r*=.06, P=.76).

4.4 Discussion

This study showed that heart rate during exercise was lower after an 8-day non-competitive amateur cycling event. Historical reviews suggested that

submaximal heart rate is increased after intensified training (Kuipers and Keizer 1988). This is still sometimes assumed in recent studies (Brink, Visscher, Coutts, et al. 2012; Schmikli et al. 2012). However, in line with our results, experimental data showed decreased heart rate (Le Meur, Hausswirth, et al. 2013; Le Meur et al. 2014), or increased power output at a given submaximal heart rate (Decroix, Lamberts, and Meeusen 2018; Lamberts et al. 2010) after training load was increased. Interestingly, the decreased heart rate in our study was observed 5 to 8 days after the intensified training period, while other studies observed a return to baseline values after such a period of time (Decroix, Lamberts, and Meeusen 2018; Le Meur et al. 2014). This suggests that homeostasis in our study was more disturbed, possibly due to a different relative increase in training load (9 fold for 8 days in our study versus 130% for 3 weeks and 208% for 8 days, respectively). Moreover, in a review by Bosquet and colleagues (2008) it was concluded that heart rate during exercise decreased after overload training lasting longer than 2 weeks, but remained unchanged after shorter periods. Our data, however, showed that heart rate was significantly lower even after only 8 days of overload training. These observations suggest that not only the duration of intensified training, but also the relative increase in training load affects the heart rate.

In line with previous work (Le Meur et al. 2014), submaximal heart rate in our study decreased both in AF and FOR athletes. Also the correlation analyses revealed no associations between the change in heart rate and performance. This suggests that the observed change in submaximal heart rate in our study is rather a general effect of intensified training (i.e. the TFL) than that it is associated with underperformance. This can possibly be explained by the finding that, despite a decreased (sub) maximal heart rate after the TFL, $\dot{V}O_2$ was unchanged at all exercise intensities. The negative association between the pre versus post TFL changes in heart rate and O_2pulse at low and medium exercise intensity suggests that $\dot{V}O_2$ was maintained through compensation by an increased stroke volume and/or arteriovenous oxygen difference. A case study from the 1970's applied the direct Fick principle to reveal that

Table 4.3. Pearson's correlation coefficients (N=26) between pre versus post TFL changes in cardiopulmonary variables, ventilatory threshold and performance (defined as peak power output). Cardiopulmonary variables were obtained at low (80 W), medium (50% worst performance) and high (100% worst performance) exercise intensity and peak values. Worst performance was determined per subject as the lowest peak power output of all tests, so that the parameters were obtained at the same absolute workload for each exercise test.

	ΔHR (beats·min^{-1})	**ΔO$_2$pulse (ml O$_2$·beat^{-1})**	**Δ$\dot{V}$O$_2$ (L·min^{-1})**	**Δ$\dot{V}$CO$_2$ (L·min^{-1})**	**ΔRER**	**Δ $\dot{V}$E (L·min^{-1})**
Low (80 W)						
ΔO$_2$pulse (ml O$_2$·beat^{-1})	**-.56****					
Δ$\dot{V}$O$_2$ (L·min^{-1})	.18	**.67****				
Δ$\dot{V}$CO$_2$ (L·min^{-1})	.31	.30	**.63****			
ΔRER	.10	**-.46***	**-.50****	.35		
ΔVentilation (L·min^{-1})	.12	.12	.26	**.76****	**.53****	
ΔVT (L O$_2$·min^{-1})	-.14	.33	.27	.02	-.32	-.01
ΔPerformance (W)	-.21	-.05	-.22	**-.40***	-.19	-.14
Medium (50% PPO)						
ΔO$_2$pulse (ml O$_2$·beat^{-1})	**-.54****					
Δ$\dot{V}$O$_2$ (L·min^{-1})	.23	**.67****				
Δ$\dot{V}$CO$_2$ (L·min^{-1})	.38	.26	**.67****			
ΔRER	.11	**-.47***	**-.42***	.38		
ΔVentilation (L·min^{-1})	.16	.12	.31	**.64****	**.39***	
ΔVT (L O$_2$·min^{-1})	-.13	.13	-.03	-.21	-.28	.15
ΔPerformance (W)	.06	-.31	-.30	**-.44***	-.21	-.37

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	ΔHR (beats·min^{-1})	ΔO$_2$pulse (ml O$_2$·beat^{-1})	Δ$\dot{V}O_2$ (L·min^{-1})	Δ$\dot{V}CO_2$ (L·min^{-1})	ΔRER	Δ $\dot{V}E$ (L·min^{-1})
High (100% PPO)						
ΔO$_2$pulse (ml O$_2$·beat^{-1})	-.30					
Δ$\dot{V}O_2$ (L·min^{-1})	.28	**.82****				
Δ$\dot{V}CO_2$ (L·min^{-1})	.28	**.48***	**.65****			
ΔRER	.07	-.30	-.26	**.54****		
ΔVentilation (L·min^{-1})	.16	.04	.14	**.56****	**.50***	
ΔVT (L O$_2$·min^{-1})	-.06	.32	.25	-.03	-.31	-.19
ΔPerformance (W)	.06	.18	.21	-.22	**-.54****	**-.47***
Peak						
ΔO$_2$pulse (ml O$_2$·beat^{-1})	-.04					
Δ$\dot{V}O_2$ (L·min^{-1})	**.41***	**.85****				
Δ$\dot{V}CO_2$ (L·min^{-1})	.39	**.62****	**.80****			
ΔRER	.04	-.26	-.23	.31		
ΔVentilation (L·min^{-1})	**.42***	.35	**.50****	**.62****	.29	
ΔVT (L O$_2$·min^{-1})	.05	.37	**.39***	.27	-.20	.04
ΔPerformance (W)	**.50****	**.44***	**.62****	**.59****	.06	**.62****

RER: respiratory exchange ratio; VT: anaerobic threshold; PPO: peak power output;

* Significant at P<.05 level; ** Significant at P<.01 level

stroke volume decreased and arteriovenous oxygen difference increased after jogging across the United States for 6 days per week, for 2.5 months (Bruce et al. 1975). Similarly, an experimental study using impedance cardiography showed a decrease in stroke volume and cardiac output, and an increase in arteriovenous oxygen difference in overreached but not in acute fatigued athletes (Le Meur et al. 2014).

In contrast to the unchanged $\dot{V}O_2$ and the ventilatory threshold, the change in respiratory exchange ratio and pulmonary ventilation at high intensity were associated with the change in performance in our study. This suggests accelerated acidosis, resulting in an increased relative effort at exercise intensities above the anaerobic threshold and, eventually, earlier fatigue. This, however, is not in line with the current literature, which suggests unchanged or slightly decreased blood lactate concentrations in athletes with decreased performance after intensified training (Urhausen and Kindermann 2002).

The cause of the observed decrease in heart rate cannot be determined from our data. Le Meur and colleagues (2014) found lower cardiac output in FOR athletes, and concluded that this was due to adrenal insufficiency. However, plasma epinephrine concentrations in that study were only decreased at the highest exercise intensities, while stroke volume and heart rate were lower at all intensities. Therefore, adrenal insufficiency cannot solely explain the lower cardiac output. Perhaps parasympathetic hyperactivity partly causes the lower heart rate, as has been demonstrated by research on heart rate variability (Le Meur, Pichon, et al. 2013) or the rate of heart rate increase (Bellenger et al. 2017) in overreached athletes. Yet, other mechanisms such as decreased density and/or sensitivity of the adrenal receptors may affect the heart rate (Steinacker et al. 2004). Additionally, cardiac damage or decreased function of the myocardium, as is shown in several prolonged endurance exercise events (Eijsvogels and Thompson 2017), might affect the stroke volume. Further research should apply simultaneous assessment of these potential mechanisms to reveal their relative contributions.

Peak heart rate was lower after the TFL. A lower peak heart rate is generally

found in overreaching (Meeusen et al. 2013). In an observational study a decreased peak heart rate during the Tour the France and Vuelta a España has been shown (Lucía et al. 2003). By means of linear regression analysis a decrease of 0.389 beats·min^{-1} per day, or 3.1 beats·min^{-1} after 8 days of racing, was estimated. This estimation shows large similarity with the 3.4 beats·min^{-1} decrease in peak heart rate observed in our study. The decreased peak heart rate in our study was not statistically different between AF and FOR, although the visit by classification interaction effect was borderline significant (P=.056). At high intensity (100% PPO) no changes between pre and post TFL were observed. Also in another study the heart rate decreased at submaximal intensity, whereas peak heart rate was only lower in FOR but not in AF athletes (Le Meur et al. 2014). These findings do not exclude the possibility that the generally observed decreased peak heart rate in overreaching is a consequence of decreased peak power output.

Our finding that heart rate decreased after intensified training, without association with the change in performance, has important consequences for training prescription and monitoring. Firstly, prescription and monitoring is often based on heart rate defined training zones (Achten and Jeukendrup 2003). However, a lower submaximal heart rate will result in underestimating the internal training load. In line with this finding, it was recently reported that (sub) maximal heart rate immediately after the Vuelta a España was lower than 1 week before the race (Rodríguez-Marroyo et al. 2017). Recalculation of the heart rate-defined training zones showed that the time in the highest training zone (i.e. above the respiratory compensation threshold) during the race was underestimated based on the difference in the heart rate response before and after the Vuelta. Secondly, several training monitoring tools, such as the Lamberts and Lambert Submaximal Cycle Test (Lamberts et al. 2011), are based on changes in heart rate at submaximal exercise intensity. Yet, our study, as well as previous data (Le Meur et al. 2014), illustrates that a decreased heart rate is a general response after an intensified training period, rather than an indicator of decreased performance. Since decreased performance is one of

the main characteristics of overreaching (Meeusen et al. 2013), and thus the primary target of training monitoring during a period of intensified exercise, submaximal heart rate seems inadequate to monitor intensified training.

Limitations

In this study we were able to evaluate cardiopulmonary exercise tests before and after intensified training in a relative large study population. The downside of our methodology, however, is that the subjects were on average older and of lower fitness level than elite athletes. This probably means that their maximal heart rate and stroke volume were different from elite athletes. The potential effect on the timing of reaching plateau values is unclear. Additionally, the applied increase in exercise volume during the Tour for Life is not expected in elite sports practice.

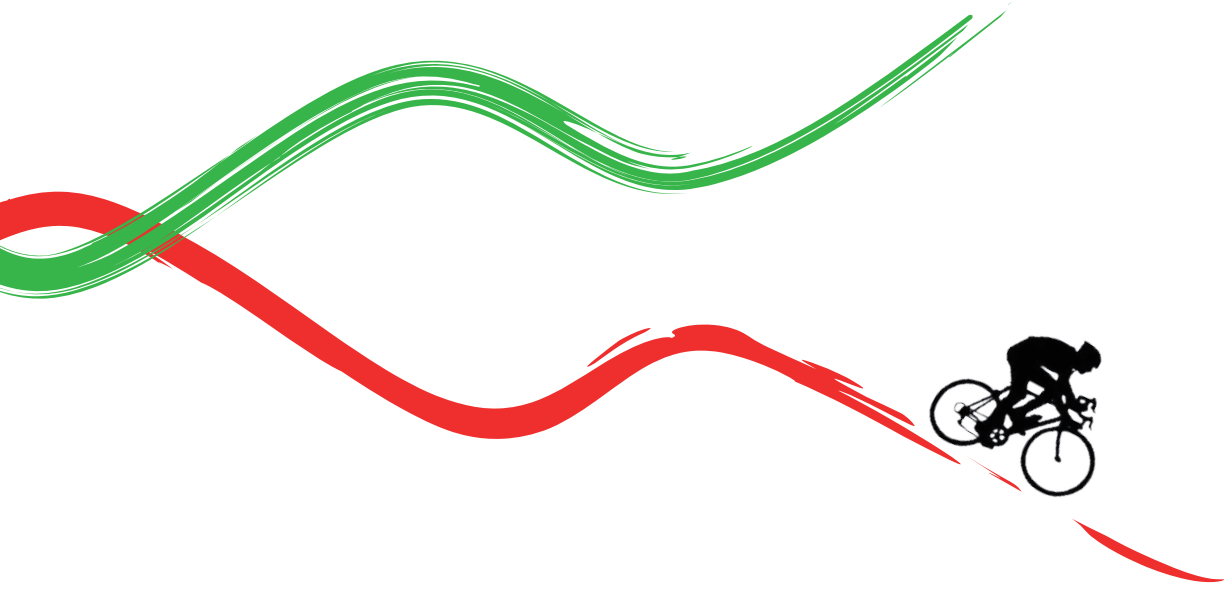
4.5 Conclusion

This study showed that heart rate at submaximal and maximal exercise intensity is lower after an 8-day non-competitive amateur cycling event. However, this decrease is unrelated to the change in performance and does not result in lower $\dot{V}O_2$. Coaches and athletes should, therefore, be aware of the limited practical value of heart rate for prescription and monitoring of intensified training.

Chapter 5

Changes in choice reaction time during and after 8 days exhaustive cycling are not related to changes in physical performance

Adapted from:
Ten Haaf, T, van Staveren, S, Iannetta, D, Roelands, B, Meeusen, R, Piacentini, MF, Foster, C, Koenderman, L, Daanen, HAM, de Koning, JJ. 2018. "Changes in choice reaction time during and after 8 days exhaustive cycling are not related to changes in physical performance." *Int J Sports Physiol Perform* 13(4): 428-33.



Abstract

Reaction time has been proposed as a training monitoring tool, but to date results are equivocal. Therefore, it was investigated whether reaction time can be used as a monitoring tool to establish overreaching. In total, 30 subjects (11 female/ 19 male, age: 40.8±10.8 y, $\dot{V}O_2$max: 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) who participated in an 8-day cycling event were recruited. The external exercise load increased approximately 900% compared to the preparation period. Performance was measured before and after the event using a maximal incremental cycling test. Subjects with decreased performance after the event were classified as functional overreached (FOR), others as acute fatigued (AF). A choice reaction time test was performed 2 weeks before (pre), 1 week after (post) and 5 weeks after (follow-up), as well as at the start and end of the event. Fourteen subjects were classified as AF and 14 as FOR (2 subjects were excluded). During the event, reaction time at the end was 68 ms (95% CI [46, 89]) faster than at the start. Reaction time post event was 41 ms (95% CI [12, 71]) faster than pre, and follow-up 55 ms faster (95% CI [26, 83]). The time by class interaction was not significant during (P=.26) and after (P=.43) the event. Correlations between physical performance and reaction time were not significant (all P>.30). To conclude, no differences in choice reaction time between AF and FOR subjects were observed. It is suggested that choice reaction time is not valid for early detection of overreaching in the field.

5.1 Introduction

An imbalance between exercise load and recovery time results in maladaptation to physical training. This process is termed overtraining, which can lead to functional (FOR) or non-functional (NFOR) overreaching or overtraining syndrome (OTS) (Meeusen et al. 2006; Meeusen et al. 2013). FOR is sometimes intentionally induced in athletes to improve performance, for example in training camps. With sufficient rest the decrease in performance is followed by supercompensation of the affected physiological systems after a couple of days to weeks. If rest is insufficient, or non-training related stress factors increase, then NFOR may occur. NFOR needs weeks to months of rest to recover. There is no clear line between normal adaptation to training and maladaptive training responses. Accordingly, it is important to monitor the training status to detect FOR early, and prevent occurrence of NFOR.

Regular exhaustive exercise tests to monitor performance do not fit in an athlete's training schedule. Therefore, sport scientists search for training monitoring tools that are easy to perform in training practice and are objective, inexpensive and not demanding (Nederhof et al. 2007). Reaction time is a measurement that fits these requirements and has been suggested as a tool for early detection of overreaching. It was introduced in the field of overreaching research because slower reaction times were found in chronic fatigue syndrome and major depression, which show symptomatic similarities with the overtraining continuum (Nederhof et al. 2006). Indeed, several studies reported slower reaction times after intensified training, confirming the possibility to detect concentration problems and cognitive complaints (Rietjens et al. 2005; Decroix, Piacentini, et al. 2016; Le Meur, Hausswirth, et al. 2013). However, some studies found no changes (Jeukendrup et al. 1992) or only a trend towards slower reaction time (Nederhof et al. 2007). It was, therefore, concluded that additional research is necessary with more subjects and a greater degree of overload (Nederhof et al. 2007; Halson 2014). We had the opportunity to follow a group of middle-aged cyclists during an 8-day charity ride, covering 1300 km with 18,500 climbing meters,

which we believe is a natural experiment likely to induce at least acute fatigue (AF) and potentially FOR or NFOR. Accordingly, the aim of this study was to investigate whether reaction time could be used as a monitoring tool to indicate FOR. It was hypothesized that directly and shortly after a training overload period reaction time would be slower in overreached subjects, but not in subjects that were able to maintain their performance level. Also, it was evaluated whether this difference would disappear after a recovery period.

5.2 Methods

Subjects

A total of 30 subjects (11 female, 19 male) were recruited amongst participants of an 8-day cycling tour. At baseline, mean±SD age was 40.8±10.8 y, BMI was 23.5±2.1 $kg \cdot m^{-2}$ and $\dot{V}O_2max$ was 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. Subjects were categorized in performance level 1 (4%), 2 (57%), 3 (25%) and 4 (14%) according to $\dot{V}O_2max$ based athlete classification norms (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016). Subjects gave written informed consent prior to the first measurements. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical committee.

Design

Subjects visited the laboratory 2 weeks before (pre), 1 week after (post) and 5 weeks after (follow-up) the Tour for Life (TFL) (Figure 5.1). The TFL is a non-competitive cycling event in which contestants cycle from Italy to the Netherlands in 8 days (1,300 km with 18,500 climbing meters). The average self-reported training volume during preparation was 144±46 km per week. The external load during the TFL was approximately 900% compared to preparation. The duration of each stage was on average 495±66 minutes and the Rating of Perceived exertion was 6.9±1.2 out of 10 (ten Haaf et al. 2017).

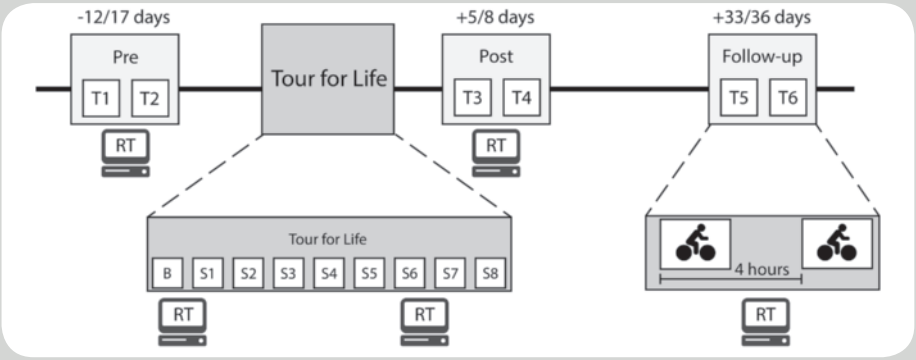


Figure 5.1. Study design. Two maximal incremental cycling tests (two bout exercise protocol (Meeusen et al. 2004)) were performed pre, post and follow-up. A choice reaction time test was performed before stage 1 and after stage 6 during the TFL, as well as in the rest period between the cycling tests each laboratory visit. T: cycling test, B: baseline TFL, S: stage.

At each laboratory visit the subjects performed a two bout exercise protocol (Meeusen et al. 2004). This protocol consists of a maximal incremental cycle ergometer test both in the morning and afternoon, with 4 hours between the starts of the exercise tests. At least 45 minutes (on average 104±31 min.) after the morning exercise test the subjects performed a choice reaction time test (see details below).

During the TFL the choice reaction time test was performed late afternoon on the day before the first stage (start), and 2 hours after the finish of stage 6 (end). For practical reasons it was impossible to perform the choice reaction time test after stage 7 or 8.

Exercise tests

Physical performance was measured using a maximal incremental cycle ergometer test until exhaustion (Excalibur Sport, Lode Medical Technology, Groningen, the Netherlands, or; Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden). The test started with a power output of 80 W for 3 minutes

after which power output increased 40 W for men and 30 W for women every 3 minutes. Subjects cycled at a freely chosen cadence. The test was stopped when subjects were unable to maintain the cadence above 60 rpm, despite strong verbal encouragement. Peak power output was defined as the average power output over the last 3 minutes of the test. Each subject performed the tests on the same ergometer and at the same time of the day throughout the study. Saddle and handle bar height were measured after the first test and the same settings were used in subsequent tests.

Choice reaction time test

Reaction time was assessed by the computerized Finger-Precuing Task (Adam et al. 1998), a test modified from the test as described by Miller (1982) (Figure 5.2). Each task started with the warning sign displayed on a computer screen: 4 plus signs on a row (+ + + +), which indicated the 4 possible target locations on the keyboard (left middle finger, left index finger, right index finger and right middle finger). After 750 ms a cue signal was presented below the warning sign. Cue signals were presented in 1 of 4 conditions: hand-cued (plus signs on the 2 left most or 2 right most positions for the left or right hand), finger-cued (the 2 outer or 2 inner positions for the middle or index fingers), neither-cued (positions 1 and 3 or 2 and 4) or uncued (plus signs on all 4 positions). The target signal was presented 500 ms after the cue signal in a position indicated by the cue. The subjects were instructed to use the cueing signal to prepare for the target sign and to press the corresponding keyboard button as fast as possible. Each test consisted of 160 tasks, 40 for each of the 4 cueing conditions in random order. Reaction time was measured by the computer as the time between the appearance of the target signal on the screen and the response on the keyboard. The test was performed with 3 subjects simultaneously in a single room, but with each other out of field of vision. Before each test a practice trial of 10 tasks was performed (Nederhof et al. 2007).

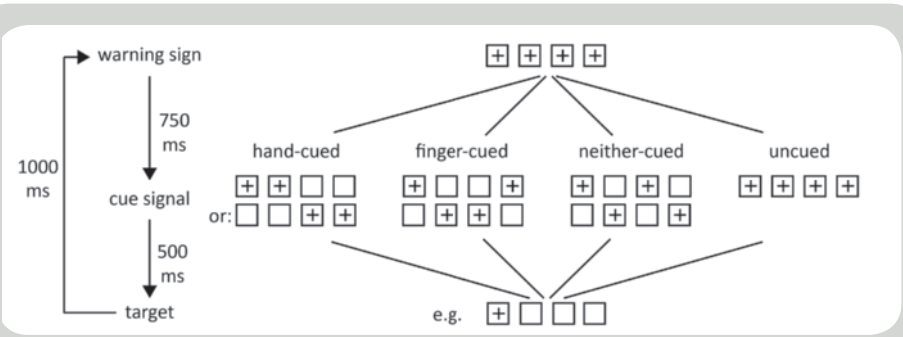


Figure 5.2. Finger-Precuing Task. Each task started with a warning sign, after 750 ms followed by a cue signal in 1 of 4 possible conditions. The cue signal was followed by a target signal after 500 ms, whereupon the subject had to press the corresponding button as fast as possible.

In accordance with previous research (Nederhof et al. 2007; Rietjens et al. 2005), tasks faster than 150 ms or slower than 1500 ms (0.09%) and incorrect tasks (1.28%) were discarded. The mean reaction time was calculated over the correct tasks for each of the 4 cueing conditions.

Classification: AF or FOR

Depending on the adaptation to the TFL, subjects were classified as either functional overreached (FOR) or acute fatigued (AF). In line with previous research (ten Haaf et al. 2017; Aubry et al. 2014; Decroix, Piacentini, et al. 2016), subjects were classified as FOR if the performance decrement in both post TFL exercise tests exceeded the smallest worthwhile change. All other subjects were classified as AF. The smallest worthwhile change was calculated as 0.3 * the coefficient of variation of peak power output between the 2 exercise tests pre TFL (T1 and T2) (Hopkins, Hawley, and Burke 1999).

Statistical analyses

Normality was checked using Shapiro-Wilks tests. Differences in baseline characteristics between AF and FOR were tested using independent t-tests, and gender distribution by a chi-square test. Differences in peak power output ($W \cdot kg^{-1}$) were analysed using a mixed design ANOVA with visit (pre, post, follow-up) and test (morning, afternoon) as within-subject factors, and class (AF, FOR) as between-subject factor. Also, a mixed design ANOVA including time (start, end TFL) and cueing condition (hand-, finger-, neither-, uncued) as within-subject factor, and class (AF, FOR) as between-subject factor was used to investigate changes in reaction time and differences between AF and FOR during the TFL. Another mixed design ANOVA included visit (pre, post, follow-up) as a within-subject factor to investigate reaction time of AF and FOR in the laboratory measurements. Significant main effects were followed-up by pairwise comparisons. Contrasts were used to further investigate significant interactions. Pearson correlation coefficients were computed between the change in reaction time and change in physical performance pre TFL versus post TFL, and pre TFL versus follow-up. Also, the correlation between change in reaction time during the TFL and the change in physical performance pre TFL versus post TFL was computed. The reaction time averaged over all cueing conditions was used in correlation analyses. Main effects and interactions were considered significant if $P<.05$.

Table 5.1. Subject characteristics (mean±SD).

	AF (N=14)	FOR (N=14)	P-value
Sex (m/f)	11/3	7/7	.12
Age (yrs)	42.6 ± 11.0	39.9 ± 10.7	.50
Body mass index ($kg \cdot m^{-2}$)	24.0 ± 1.7	23.2 ± 2.3	.30
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.3 ± 6.2	51.8 ± 6.8	.90
Peak power ($W \cdot kg^{-1}$)	4.15 ± 0.61	4.05 ± 0.53	.62

AF: acute fatigued, FOR: functional overreached

Statistical analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 23.0, Amonk, NY).

5.3 Results

Subjects

One subject was excluded because of asthmatic symptoms during the exercise tests. Another subject was excluded from analyses because of a mild traumatic brain injury during preparation for the TFL (Belanger et al. 2005). The coefficient of variation of incremental cycling performance pre TFL was 1.6%, meaning a smallest worthwhile change of 0.5%. This resulted in 14 subjects classified as FOR and 14 as AF. The baseline characteristics of the AF and FOR subjects are displayed in Table 5.1. No significant differences were observed for age or fitness level. Pre TFL data of 2 subjects, and end TFL data of 1 subject were missing due to technical errors. Two subjects dropped-out after the post TFL measurements due to injuries. These subjects were excluded from analyses that involved follow-up data.

Physical performance

When considering the whole group, the peak power output was significantly

Table 5.2. Peak power output ($W \cdot kg^{-1}$, mean±SD) for acute fatigued and functional overreached subjects.

	Acute fatigue			Functional overreaching		
	1st test	2nd test	average	1st test	2nd test	average
Pre TFL	4.17±0.65	4.08±0.59	4.13±0.62	4.07±0.58	4.09±0.56	4.08±0.57
Post TFL	4.16±0.61	4.14±0.58	4.15±0.59	3.84±0.58*	3.91±0.59*	3.87±0.58*
Follow-up	4.05±0.61	4.11±0.55	4.08±0.57	3.93±0.47	3.94±0.48	3.94±0.47

* Significant ($p<.05$) different from Pre

different between the visits (P=.02, η_p^2=.15), with the post TFL power output being 0.09 W·kg^{-1} (95% CI [0.01, 0.17]) lower than pre TFL. The peak power output measured post TFL was significantly (P<.001, η_p^2=.39) more decreased in FOR (pre: 4.08±0.57, post: 3.87±0.58 W·kg^{-1}) than in AF (pre: 4.13±0.62, post: 4.15±0.59 W·kg^{-1}), without differences between the morning and afternoon exercise test. This difference between AF and FOR followed from the distinction based on physical performance. Details are shown in Table 5.2.

Reaction time

During the event, reaction time at the end was on average 68 ms faster than at the start (P<.001, η_p^2=.62). In the laboratory tests, reaction time was faster (P<.001, η_p^2=.44) in the successive visits. Reaction time improved from pre TFL to post TFL by 41 ms (95% CI [12, 71]) and was fastest at follow-up (55 ms faster than pre TFL, 95% CI [26, 83]). The reaction time for the hand-cued condition was faster than in the other conditions (all P<.001), whereas the uncued condition resulted in slower reaction times (all P<.05).

Relation physical performance and reaction time

During the TFL, no significant effect was found for the time by class (P=.26, η_p^2=.05) or time by cueing condition by class interaction (P=.39, η_p^2=.04). Also in the laboratory measurements, neither the visit by class (P=.43, η_p^2=.03), nor the visit by condition by class interaction (P=.98, η_p^2=.01) were significant. This means that the change in reaction time during the TFL and in the laboratory measurements was not different between AF and FOR. The reaction time averaged over all cueing conditions for AF and FOR is displayed in Figure 5.3.

Correlations between the change in reaction time and change in performance were not significant for pre versus post TFL (*r*=.17, P=.41) and pre versus

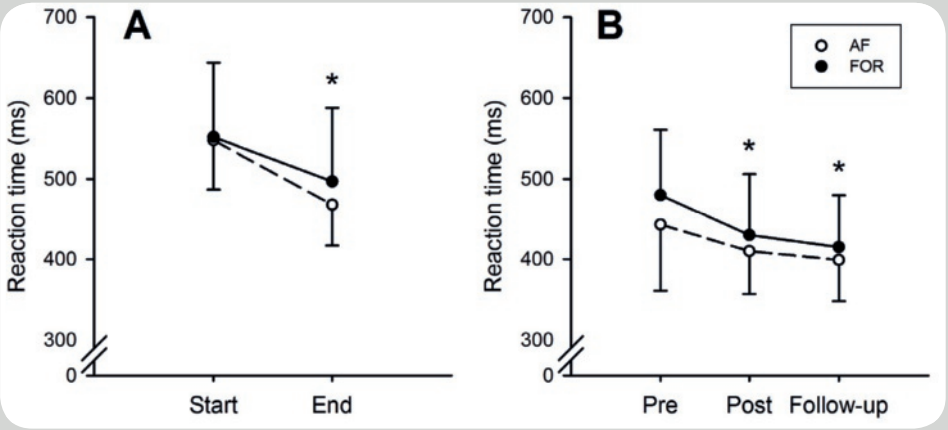


Figure 5.3. Mean±SD reaction time (ms) during Tour for Life (**A**) and in pre-, post- and follow-up laboratory measurements (**B**). Values are averaged over all cueing conditions. AF: acute fatigue, FOR: functional overreaching, *Significant (p<.05) different from Start/Pre.

follow-up (*r*=-.21, P=.32). Also, the correlation between change in reaction time during the TFL and pre versus post TFL change in physical performance was not significant (*r*=-.15, P=.44).

5.4 Discussion

The purpose of this study was to investigate whether reaction time can be used to detect overreaching. The results of this study clearly showed that changes in reaction time during or after an 8-day overload period could not distinguish FOR from AF, provided this classification was based on changes in physical performance.

Two observations stand out regarding the absolute reaction times observed in our study. Firstly, the reaction times in our study were slower than in the studies that applied the same Finger-Precuing Task (Rietjens et al. 2005; Nederhof et al. 2007). This can probably be explained by differences in age. Subjects in our study were older (40.8±10.8 y) than in other studies (25.3±4.7 y and

25.3±4.1 y) (Nederhof et al. 2007; Rietjens et al. 2005). It has been shown that reaction time is slower in middle-aged than in young adults (Adam et al. 1998; Deary, Liewald, and Nissan 2011).

Secondly, subjects in our study had a slower reaction time at the start of the TFL compared to the pre TFL laboratory measurement. It was expected that reaction time improved due to repeated performance of the test (Rietjens et al. 2005; Decroix, Piacentini, et al. 2016; Nederhof et al. 2007). A methodological difference was that the pre TFL laboratory measurements were performed 98±23 minutes after an exercise test, whereas the test at the start of TFL was performed on a resting day. It is well established that reaction time improves due to acute exercise (Lambourne and Tomporowski 2010). However, this effect is largest 10-20 minutes after exercise, and diminishes thereafter (Chang et al. 2012). Hence, it is unlikely that the slower reaction time at the start of the TFL results from (the absence of) preceding exercise. In contrast to very well controlled laboratory measurements, many factors are of potential influence on reaction time in field measurements. For example, sleep quality was reduced during the TFL (ten Haaf et al. 2017). Because reaction time was slower in the field than in the laboratory measurements, these settings were analysed separately. Therefore, we focussed in this study on the improvement in reaction time both during the TFL and throughout the laboratory measurements.

Reaction time has been described as a promising tool to monitor training status (Meeusen et al. 2013). However, this study does not provide support for this hypothesis. In our study, reaction time became faster throughout the laboratory measurements and during the TFL. Moreover, no difference in change in reaction time between AF and FOR was observed. It is therefore suggested that choice reaction time cannot be used to distinguish between AF and FOR in middle-aged non-professional cyclists. Among the studies on reaction time that compared FOR to AF athletes, 2 studies (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010) concluded that reaction time became slower in FOR while AF subjects became faster. On the other hand,

Nederhof et al. (2007) did not find significant differences between FOR and AF athletes. Two studies compared FOR with controls that continued normal training. Those studies reported reduced improvement in reaction time (Rietjens et al. 2005) or slower reaction time only at exhaustion but not at rest (Le Meur, Hausswirth, et al. 2013).

Several differences between the studies can possibly account for different results. Firstly, slightly different criteria were used to define overreaching. Similar to our study, some studies applied the smallest worthwhile change in performance as a cut-off value (Le Meur, Hausswirth, et al. 2013; Decroix, Piacentini, et al. 2016), whereas Dupuy et al. (2010) defined all subjects with a decreased physical performance as FOR. Nederhof et al. (2007) distinguished between AF and FOR based on at least 10 watts decrease in performance together with changes in hear rate, $\dot{V}O_2$max and mood. It seems, however, unlikely that variations in cut-off values account for different findings between our and other studies, since correlation analyses in our study revealed no relation between physical performance and reaction time.

Secondly, different tests were applied to measure reaction time. In our study the Finger-Precuing Task was used. Simple reaction time tests concern only attention, whereas performance on the Finger-Precuing Task depends more on post-perceptual processing (Adam et al. 1998). The different cueing conditions can help in selective preparation of the target signals and enhance post-perceptual processing time. This was confirmed by our results, which showed that reaction time was fastest for the hand-cued condition and slowest for the uncued condition. Reaction time improved more for the neither-cued condition than for the other cueing conditions. It has been pointed out in literature that research is necessary to find out which test is most sensitive to measure changes in training status. Besides the Finger-Precuing Task (Rietjens et al. 2005; Nederhof et al. 2007), results for the modified Stroop test (Dupuy et al. 2010; Decroix, Piacentini, et al. 2016) and a simple reaction time test (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010; Le Meur, Hausswirth, et al. 2013) were reported in literature, but with conflicting results for every test.

Therefore, it is unlikely that the different tests applied to measure reaction time account for different findings between studies.

A third factor that can possibly explain different results between studies is the difficulty of the applied test. Dupuy et al. (Dupuy et al. 2010) and Le Meur et al. (Le Meur, Hausswirth, et al. 2013) suggested that in accordance with the threshold theory (Satz 1993), reaction time was only affected in FOR beyond a threshold of cognitive effort. Le Meur et al. (Le Meur, Hausswirth, et al. 2013) evaluated simple reaction time during exercise, and only at exhaustion the reaction time was different between FOR and control subjects, whereas at easier testing conditions (rest or submaximal intensity) no differences were found. In line with these findings, only in the most difficult condition of the Stroop test differences between positive and negative adapters to overload training were observed in another study (Dupuy et al. 2010). Similarly, underperforming athletes made more mistakes than control subjects at moderate or high speeds, but not at slow speed in a modified Stroop test (Hynynen et al. 2008). In contrast to these studies, Nederhof et al. (Nederhof et al. 2007) did not find significant differences in the Finger-Precuing Task, nor were effects found in a determination test that was considered more difficult. In addition, a recent study of Decroix et al. (Decroix, Piacentini, et al. 2016) described differences in a simple reaction time test, but not in the more difficult Stroop test. These conflicting results suggests that not the difficulty of the reaction time test per se affects the outcome of the study, but that inclusion of different reaction time tests possibly resulted in increased chances for false positive findings. Indeed, in a meta-analysis on reaction time an effect of reporting multiple tests was found, indicating that inclusion of multiple reaction time tests was a source of bias (Lambourne and Tomporowski 2010). Thus, different findings between our study and other studies may arise from an increased type I error.

Another factor that could possibly account for different findings between studies was the increase in training load. Following the advice of Nederhof et al. (Nederhof et al. 2007), more subjects were included and greater overload

was applied in our study. The training volume in this study was approximately 900% of the volume during the preparation period, whereas 140% to 200% of baseline load was used in other studies (Rietjens et al. 2005; Dupuy et al. 2010; Dupuy et al. 2014; Le Meur, Hausswirth, et al. 2013; Decroix, Piacentini, et al. 2016). Though, the overload in our study should be viewed in the context of the nature of the overload period. The cycling tour in this study was not a training camp in which athletes deliberately go in functional overreaching. Instead, the subjects might have cycled some parts of the stages at relative easy pace. Still, the internal training load was very high, as indicated by subjective ratings. It should be acknowledged that the overload in our study is not to be expected in (elite) sports. Therefore, a limitation of this study is that our data cannot be generalized to all training conditions in sports practice. However, even with the enormous overload in this study, an improvement in reaction time was observed, without differences between AF and FOR subjects.

Practical application

The results of this study suggest that, in contrast to previous research, reaction time is not a sensitive tool to monitor athletes' training status. However, non-competitive cyclists were included in this study, and results do not necessarily apply to professional athletes. Also, it should be emphasized that in a field test, compared to very strict experimental conditions in a laboratory test, several factors can influence the subject. This may explain differences between studies, but is also valuable practical information for trainers and coaches who most likely test athletes in field settings. Therefore, it is suggested that athlete support staff does not rely on reaction time to track their athletes' fitness.

5.5 Conclusion

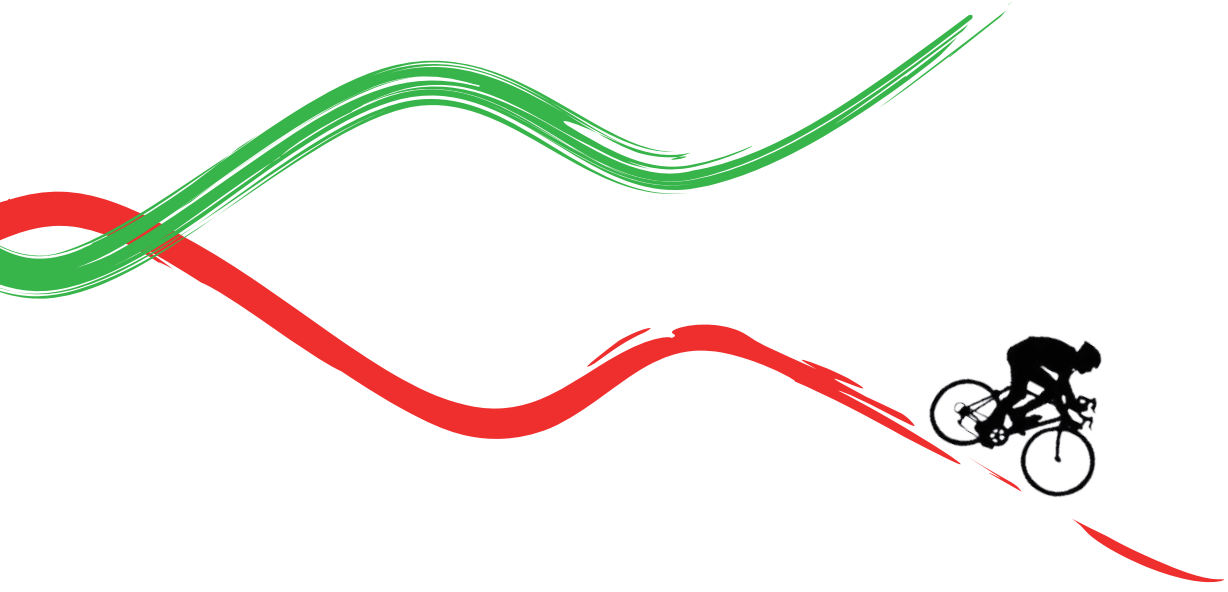
It was hypothesized that reaction time would be slower in FOR subjects, but

not in AF subjects after a training overload period. Our results do not provide support for this hypothesis. In contrast, it was shown that the change in performance on a choice reaction time test during or after 8 days exhaustive exercise was not different between acute fatigued and functional overreached subjects, classified according to change in physical performance. It is therefore suggested that reaction time is not valid for early detection of overreaching in the field.

Chapter 6

Prediction of functional overreaching from subjective fatigue and readiness to train after only 3 days of cycling

Adapted from
Ten Haaf, T, van Staveren, S, Oudenhoven, E, Piacentini, MF, Meeusen, R, Roelands, B, Koenderman, L, Daanen, HAM, Foster, C, de Koning, JJ. 2017. "Prediction of functional overreaching from subjective fatigue and readiness to train after only 3 days of cycling." *Int J Sports Physiol Perform* 12 (Suppl 2): 287-94.



Abstract

The aim of this study was to investigate whether monitoring of easily measurable stressors and symptoms can be used to early distinguish between acute fatigue (AF) and functional overreaching (FOR). We recruited 30 subjects (11 female/ 19 male, age: 40.8±10.8 y, $\dot{V}O_2$max: 51.8±6.3 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) who participated in an 8-day cycling event over 1,300 km with 18,500 climbing meters. Performance was measured before and after the event using a maximal incremental test. Subjects with decreased performance after the event were classified as FOR, others as AF. Mental and physical well-being, internal training load, resting heart rate, temperature and mood were measured daily during the event. Differences between AF and FOR were analysed using mixed model ANOVAs. Logistic regression was used to determine the best predictors of FOR after three and six days of cycling. Fifteen subjects were classified as FOR and 14 as AF (one excluded). Although total group changes were observed during the event, no differences between AF/FOR were found for individual monitoring parameters. The combination of changes in subjective ratings of fatigue and readiness to train after three days cycling correctly predicted 78% of the subjects as AF or FOR (sensitivity=79%, specificity=77%). It was concluded that monitoring changes in fatigue and readiness to train, using simple visual analogue scales, can be used to identify subjects likely to become FOR after only 3 days of cycling. Hence, we encourage athlete support staff to not only monitor fatigue, but also the subjective integrated mental and physical readiness to perform.

6.1 Introduction

Overtraining is a process of intensified training and insufficient rest, with possible outcomes of functional (FOR) or non-functional overreaching (NFOR) or overtraining syndrome (OTS) (Meeusen et al. 2006; Meeusen et al. 2013). FOR might be a normal part of the fitness-fatigue adaptive continuum and is often deliberately induced in training camps to improve performance. However, it has been demonstrated that the supercompensation effect of performance was smaller for FOR than compared to acute fatigued (AF) athletes after a 3-week training camp (Aubry et al. 2014; Aubry et al. 2015). Also, it was shown that FOR athletes experienced disturbed sleep and had a higher incidence of illness compared to AF (Hausswirth et al. 2014). Moreover, FOR precedes NFOR/OTS, which is the part of the overtraining continuum where the more severe and persistent symptoms such as psychological distress and/or endocrine disturbances occur (Meeusen et al. 2006; Meeusen et al. 2013). Prevention of NFOR/OTS is therefore of utmost importance. For these reasons, athletes and coaches need to be aware of FOR during competition and training camps. Hence, the focus of this study is on the distinction between AF and FOR in order to be able to early identify FOR.

Since there is no known single cause and mechanism of the overtraining continuum, monitoring of stressors and symptoms seems essential for prevention. Monitoring tools must be easy to use, inexpensive, give quick results and be able to sense changes before symptoms occur (Meeusen et al. 2006; Meeusen et al. 2013). Training load, mood disturbances, resting heart rate and the combination of heart rate recovery with perceived fatigue fit these criteria and are often used in monitoring studies (Bosquet et al. 2008; Brink et al. 2010; Comotto et al. 2015; Le Meur et al. 2017). Monitoring of the external training load, quantified for example by distance or power output, is often insufficient because many circumstances can influence the ability of an athlete to handle a given external load (Halson 2014). The internal load is the physiological and psychological stress that results from the external load.

Studies on the overtraining spectrum generally have cross-sectional designs with small sample sizes, because the impact of symptoms and lack of effective therapies make it hard to apply overtraining as an intervention (Armstrong and VanHeest 2002). These studies do not provide insight in the development of the overtraining continuum. Therefore, the aim of this study was to investigate whether monitoring of easily measurable stressors and symptoms can be used to early distinguish between AF and FOR.
In this study the development of FOR was investigated in amateur cyclists who participated in the Tour for Life (TFL). The TFL is an 8-day recreational fundraising cycling tour from Italy to The Netherlands (1,300 km with 18,500 climbing meters) involving mostly middle-aged athletes. The TFL model allowed us to study the development of FOR in a relatively large population.

6.2 Methods

Subjects

Thirty (11 female, 19 male) recreational cyclists participated in the study (mean±SD age 40.8±10.8 y, BMI 23.5±2.1 $kg \cdot m^{-2}$). At baseline the $\dot{V}O_2max$ was 51.8±6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ and peak power output 4.12±0.57 $W \cdot kg^{-1}$. Subjects reported an estimated training volume of 144±46 km per week during preparation. The subjects were categorized in performance level 1 (4%), 2 (57%), 3 (25%) and 4 (14%) according to the $\dot{V}O_2max$ based athlete classification norms (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016). The subjects were relatively fit for their age, reflected by a $\dot{V}O_2max$ of 148±17% relative to the Jones norm (1985).
An introductory meeting was organized during which the purpose, study design and measurements were explained to the subjects. Before the first measurements, subjects gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethical committee.

Table 6.1. External and internal training load per stage.

Stage		Distance (km)	Climbing (m)	Duration (min)	sRPE (AU)	Load (AU*10³)
1	AF	110	2,800	389±62	7.9±1.0	3.1±0.7
	FOR			386±68	7.6±1.5	2.9±0.7
2	AF	160	4,200	616±75	7.4±2.1	4.6±1.5
	FOR			569±146	7.9±1.8	4.4±1.4
3	AF	175	2,900	586±88	7.1±1.8	4.3±1.5
	FOR			543±163	6.8±2.7	4.0±1.8
4	AF	190	1,900	555±80	7.3±2.4	4.1±1.6
	FOR			509±159	5.7±2.7	3.2±1.8
5	AF	129	2,450	405±137	5.3±2.4	2.3±1.1
	FOR			434±80	6.9±1.4*	3.0±1.0
6	AF	175	900	417±62	6.0±2.1	2.5±0.9
	FOR			411±59	5.5±2.4	2.2±1.0
7	AF	215	2,200	554±70	7.1±1.9	4.0±1.2
	FOR			561±96	7.7±1.2	4.3±0.9
8	AF	110	1,200	No data available		
	FOR					
Total	AF	1,264	18,550	3522±397	6.9±1.5	24.8±6.7
	FOR			3414±523	6.9±1.0	24.1±5.7

Note: for practical reasons no data could be collected after stage 8.
AF: Acute fatigue; FOR: functional overreaching
* P<.05 for difference between AF and FOR

Design

This observational study was designed around the TFL. The subjects cycled together with about 400 contestants from Italy to the Netherlands in 8 days. They slept in tents at camping sites. The start of the stages was between 0700h and 0800h and the finish between 1600h and 1930h, depending on the rider's fitness level and the length of the stage. The external training load of each stage is shown in Table 6.1. Every morning resting heart rate and rectal temperature were measured. Within 30 minutes of finishing stages 1 to 7 subjects reported the session Rating of Perceived Exertion (sRPE) (Foster et al. 1995; Christen et al. 2016) and completed a 7-item diary (Piacentini and Meeusen 2015) on subjective physical and mental well-being. It was practically impossible to collect data after this last stage. The 32-item Dutch version of the Profile of Mood States questionnaire (POMS) (Wald and Mellenbergh 1990) was filled out the evening before the first stage (start), and after stage 3 (halfway) and

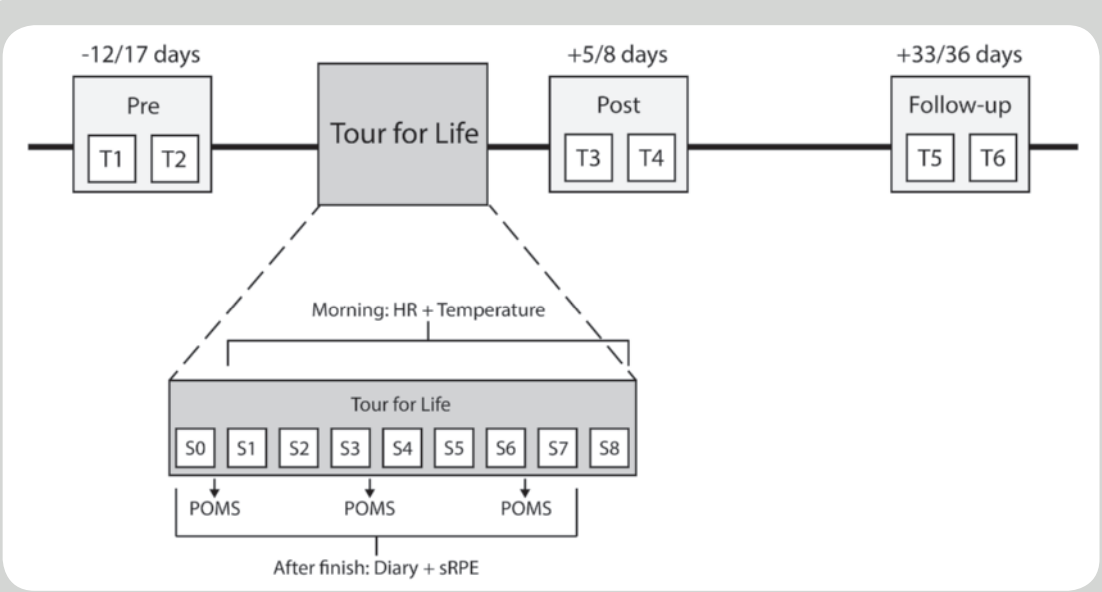


Figure 6.1. Study design. Two maximal incremental tests were performed before and twice after an 8-day cycling event. T: Maximal incremental tests; S: stage; HR: resting heart rate; POMS: Profile of Mood States; sRPE: session Rating of Perceived Exertion.

stage 6 (end) of the TFL.
Subjects visited the laboratory 12-17 days before, and 5-8 days and 33-36 days after the TFL (Figure 6.1). During each laboratory visit the subjects performed two maximal incremental tests on a cycling ergometer with 4 hours between the start of both tests (Meeusen et al. 2004).

Exercise tests

The laboratory exercise tests started with cycling (Excalibur Sport, Lode Medical Technology, Groningen, The Netherlands, or; Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden) at 80 W for 3 minutes. Power increased 40 W for men and 30 W for women every 3 minutes. The subjects cycled at a freely chosen pedalling frequency. The test was stopped when subjects were unable to maintain a pedalling frequency above 60 rpm, despite strong verbal encouragement. Peak power output was defined as the average power output over the last 3 minutes of the exercise test. Each subject performed the tests on the same ergometer and at the same time of the day. Saddle and handle bar height were measured after the first test and the same settings were used in subsequent tests.

Monitoring of stressors and symptoms

Subjects rated their physical and mental well-being by means of a visual analogue scale (VAS) for 7 items after stage 1 to 7. Items included 'sleep quality', 'general mental well-being', 'general physical well-being', 'readiness to train', 'muscle soreness', 'fatigue' and 'attractiveness of the training day' (Piacentini and Meeusen 2015).
sRPE was determined by asking individuals to rate the intensity of the stage on a VAS. sRPE and exercise time were used to calculate load, monotony and strain (Foster 1998). The product of the sRPE (0-10) and stage duration (in minutes) was termed the daily load. Total load was calculated for the first

half of the TFL (stage 1 to 3) and for all stages by summation of the daily loads. Monotony and strain were calculated over all stages. Monotony was computed by dividing the average daily load by the SD of the daily load. Strain was calculated as the product of total load and monotony.
Resting heart rate was measured with the subject's own heart rate monitor and rectal temperature with a standard commercial thermometer every day during TFL directly after waking up. The validity of the thermometers was checked using a Pt100 digital temperature indicator (P650, Dostmann Electronic, Wertheim-Reicholzheim, Germany).
POMS subscales (vigour, anger, fatigue, depression, tension) were calculated. In addition total mood disturbance (TMD) was calculated by the sum of anger, fatigue, depression and tension and subtracting the vigour score. Also, the energy index (EI) was calculated by subtracting fatigue from vigour (Kenttä, Hassmén, and Raglin 2006).
Changes between start and halfway TFL, and between start and end TFL were calculated for POMS, 7-item diary, resting heart rate and core temperature. All monitoring data was collected using paper and pencil and digitalized using double entry method.

Classification: AF or FOR

FOR is characterized by a temporary performance decrement. In line with previous research the smallest worthwhile change (SWC) was used as a FOR threshold (Aubry et al. 2015; Aubry et al. 2014). The SWC was calculated by 0.3 * the coefficient of variation (Hopkins, Hawley, and Burke 1999) of peak power output between the two exercise tests before the TFL (T1, T2). The pre versus post TFL performance decrement was used because 1) athletes are able to recover from FOR in days to weeks, and 2) the training volume was not set in the follow-up period. Subjects whose pre versus post performance decrement was larger than the smallest worthwhile change on both exercise tests (i.e. T1-T3>T1*SWC as well as T2-T4>T2*SWC) were classified as

FOR. All other subjects were classified as acute fatigued (AF).

Statistical analyses

Missing values of subjects with maximally one missing value per monitoring tool were imputated. For the POMS the missing value was replaced with the individual mean of the subscale. For the other measures the ratio between the subject's value and the other subjects for the non-missing stages was used. In total 44 out of 5250 data entries were imputated (0.8%).

Differences in total load, monotony and strain were assessed using independent t-tests. Mixed-model analyses of variance with stage as a within-subject factor and class (AF/FOR) as a between-subjects factor were performed for the change in peak power output and all daily monitoring measures. Interactions were followed-up with one-way ANOVAs if they were significant at the 5% level.

Binary logistic regression analysis was carried out to identify a combination of monitoring measures that predict FOR. Analysis was carried out for changes halfway and for changes between start and end of the TFL. Firstly, a preselection of predictors was performed by testing all monitoring measures individually in logistic regression (i.e. diary items, load, monotony, strain, resting heart rate, rectal temperature, POMS subscales, TMD, EI). The three measures with the lowest P-value for the Wald statistic were together inserted in a backward method logistic regression with class (AF/FOR) as the outcome variable. Assumptions of linearity and multicollinearity of the predictors were checked and residuals were analysed for outliers and influential cases. Magnitude-based inferences were described as advised by Hopkins and colleagues (Hopkins et al. 2009). Statistical analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 23.0. Amonk, NY).

Table 6.2. Subject characteristics (mean±SD).

	AF (N=14)	FOR (N=15)	P-value
Sex (m/f)	11/3	7/8	.13
Age (yrs)	42.6 ± 11.0	39.1 ± 10.7	.39
Body mass index ($kg \cdot m^{-2}$)	24.0 ± 1.7	23.0 ± 2.3	.22
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.3 ± 6.2	51.8 ± 6.6	.83
Peak power ($W \cdot kg^{-1}$)	4.15 ± 0.61	4.06 ± 0.52	.64

AF: acute fatigued, FOR: functional overreached

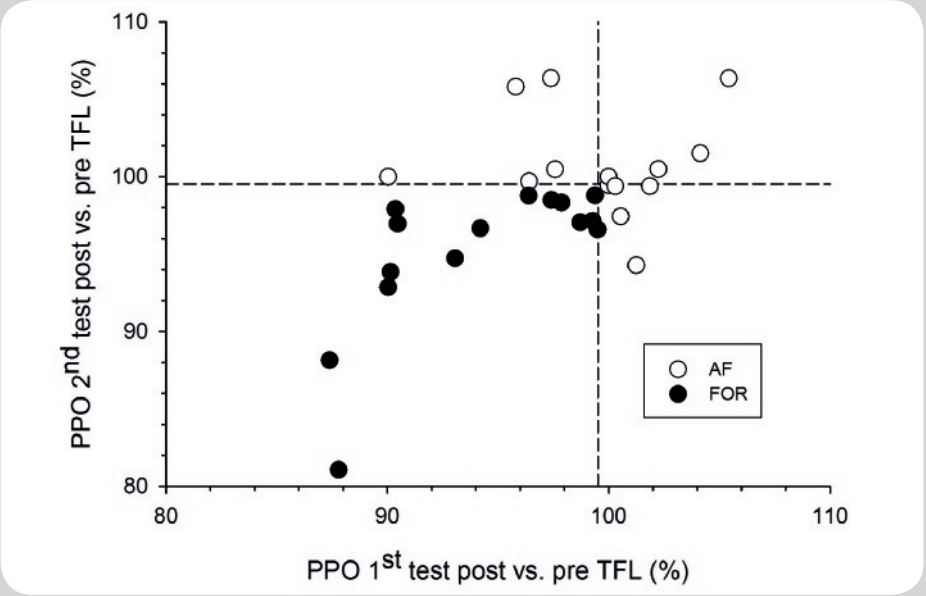


Figure 6.2. Classification of Acutely Fatigued (AF) and Functionally Overreached (FOR) subjects. Subjects who's performance decrement on both (T1 vs. T3 and T4 vs. T2, Fig. 1) exercise tests was larger than the smallest worthwhile change (dashed lines) were classified as FOR. PPO: Peak Power Output, TFL: Tour for Life.

6.3 Results

Subjects

One subject was excluded from analysis because of asthmatic symptoms during the post TFL exercise tests. The coefficient of variation of peak power output during pre TFL exercise tests was 1.6%, so the SWC was 0.5%. Based on the described criteria this resulted in 15 subjects being classified as FOR and 14 as AF (Figure 6.2). Characteristics of the classes are displayed in Table 6.2.

AF versus FOR

The performance decrement post TFL was almost certainly larger for

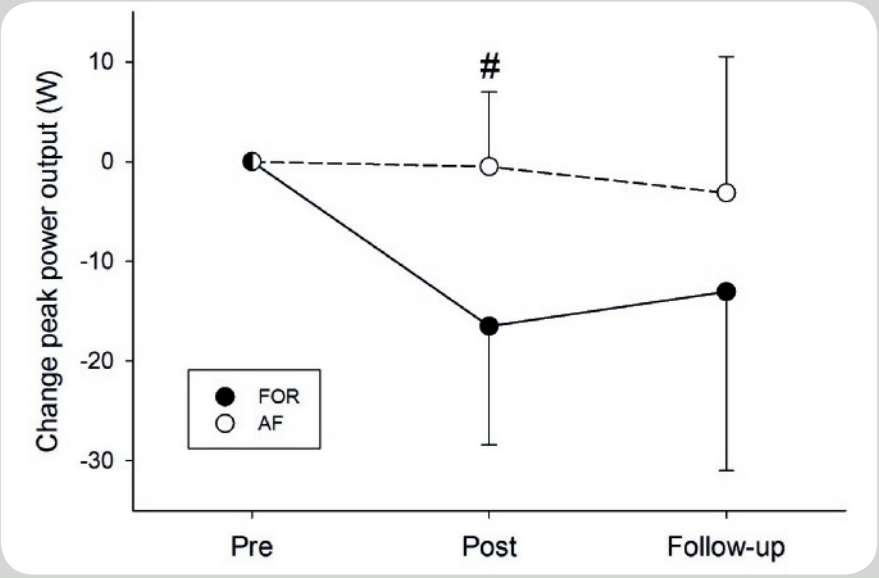


Figure 6.3. Change in peak power output for the acute fatigued (AF) and functional overreached (FOR) subjects (mean±SD). #: different from FOR (P<.05).

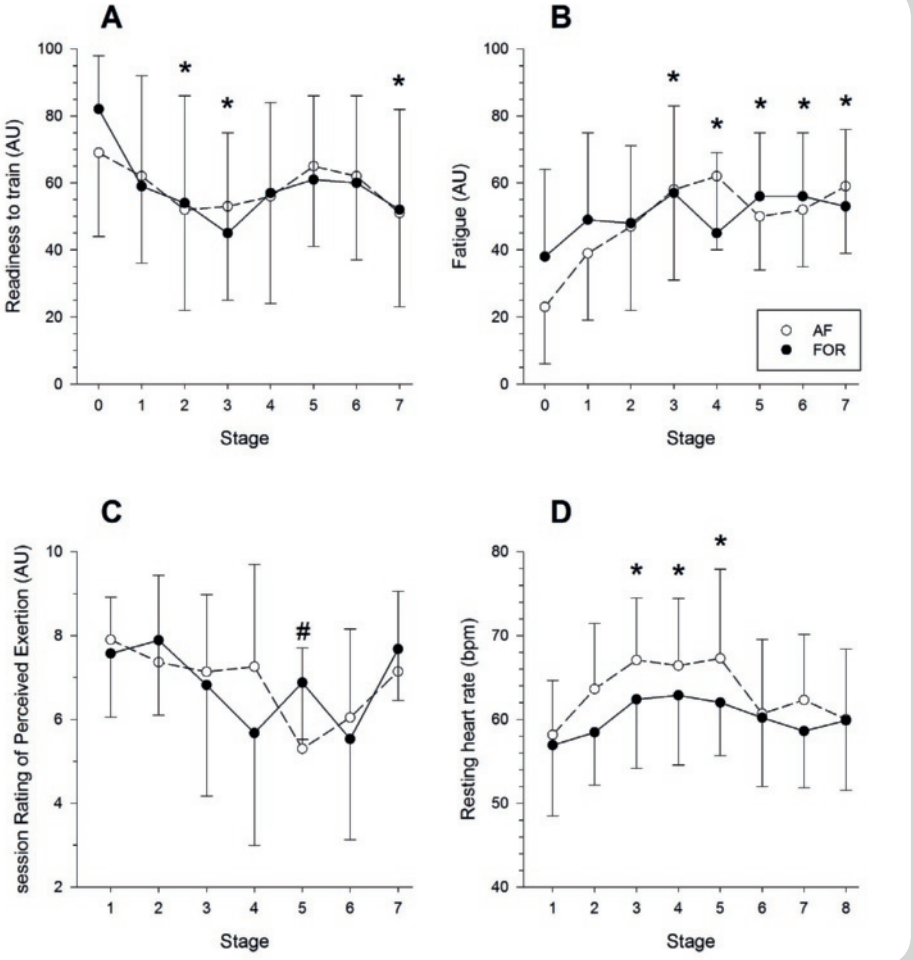


Figure 6.4. Readiness to train (**A**), fatigue (**B**), session Rating of Perceived Exertion (**C**) and resting heart rate (**D**) for acute fatigued (AF) and functional overreached (FOR) subjects per stage of the Tour for Life (mean±SD). #: different from FOR, *: different from baseline (P<.05).

FOR (-16.5±11.9 W) compared to AF (-0.5±7.5 W) (P<.01, η_p^2=0.41), and probably still larger at follow-up (-13.0±18.0 W versus -3.1±13.6 W respectively, P=.12, η_p^2=0.09) (Figure 6.3).

On a group level, readiness to train was significantly lower after stages 2, 3 and 7 compared to baseline (Figure 6.4 A). Subjects reported higher fatigue scores than at baseline from stage 3 to 7 (Figure 6.4 B). Muscle soreness after all stages was higher than at baseline. However, none of the diary items revealed a difference between AF and FOR (data not shown).

Total cycling time of stage 1 to 7 was 57.8±7.7 hours. The only significant difference between the means of FOR and AF was in the sRPE at stage 5 (6.9±1.4 AU versus 5.3±2.4 AU respectively, P=.04) (Figure 6.4 C). There were no differences between AF and FOR in total load (2.5*104±0.7*104 AU versus 2.4*104±0.6*104 AU, P=.75), monotony (3.6±1.2 AU versus 3.0±1.1 AU, P=.19) and strain (9.1*104±3.6*104 AU versus 7.5*104±3.2*104 AU, P=.22).

Resting heart rate was significantly higher for the total group in stages 3, 4 and 5 but returned to baseline level near the end of the TFL (Figure 6.4 D). No differences between AF and FOR were observed. The observed effect of class on rectal temperature was probably small (AF: 35.9±0.1°C, FOR: 36.3±0.1°C, P=.06, η_p^2=0.14) and did not change during the TFL (data not shown).

None of the POMS scales revealed a difference between AF and FOR (Table 6.3). On total group level, fatigue, anger and total mood disturbance increased significantly during the TFL. Vigour, tension and energy index decreased significantly after the start.

Logistic regression analyses

A logistic regression model (Table 6.4) including change in fatigue and readiness to train (both VAS) halfway through the TFL predicted 78% of the subjects in the correct class (sensitivity=79%, specificity=77%). The individual

Table 6.3. Profile of Mood States subscale scores (mean±SD).

		Start	Halfway	End
Vigour	AF	19.0±2.7	17.6±4.5	17.1±3.8
	FOR	18.0±3.3	17.3±3.5	16.3±4.4
	Total	18.5±3.0	17.4±4.0	16.7±4.1 [a]
Anger	AF	9.6±3.2	11.3±5.1	9.2±2.4
	FOR	7.9±1.5	10.0±3.2	9.8±4.1
	Total	8.8±2.6	10.6±4.2 [a]	9.5±3.3
Fatigue	AF	8.0±2.4	16.1±5.4	13.3±3.7
	FOR	9.1±4.4	14.3±4.5	11.9±3.4
	Total	8.6±3.6	15.1±5.0 [a]	12.6±3.5 [a,b]
Depression	AF	8.4±0.8	8.7±1.1	8.7±1.6
	FOR	8.5±1.1	9.2±1.6	9.4±2.2
	Total	8.5±0.9	9.0±1.4	9.1±1.9
Tension	AF	10.8±3.7	7.9±1.9	6.9±1.3
	FOR	10.4±3.1	7.1±1.8	7.1±1.8
	Total	10.6±3.4	7.5±1.9 [a]	7.0±1.5 [a]

AF: acutely fatigued; FOR: functional overreached

a: Different from start (P<.05), b: Different from halfway (P<.05)

data points for these two factors are shown in Figure 6.5. The sensitivity and specificity were lower when only fatigue (67%, 36%) or readiness (60%, 71%) was inserted in the analysis.

At the end of the TFL, change in POMS fatigue and readiness to train (VAS) predicted 75% of the subjects in the right class (sensitivity=79%, specificity=71%). The sensitivity and specificity were lower for fatigue (73%, 57%) or readiness (60%, 57%) individually.

Table 4. Predictors derived from logistic regression analysis with delta scores halfway (**A**) and end of the Tour for Life (**B**). Two subjects with multiple missing data were excluded from analysis, resulting in N=27.

A	**β (SE)**	**OR [95% CI]**	**P-value**
Constant	-0.54 (0.79)		
Δ Fatigue (VAS)	-0.08 (0.03)	0.93 [0.87 0.99]	.03
Δ Readiness	-0.10 (0.04)	0.90 [0.83 0.98]	.02

Note: R^2 = .42 (Cox & Snell), .56 (Nagelkerke), model $\chi^2(2)$ = 14.78, P<.01

B	**β (SE)**	**OR [95% CI]**	**P-value**
Constant	0.52 (0.65)		
Δ Fatigue (POMS)	-0.28 (0.14)	0.76 [0.58 0.99]	.04
Δ Readiness	-0.05 (0.02)	0.95 [0.91 1.00]	.04

Note: R^2 = .24 (Cox & Snell), .32 (Nagelkerke), model $\chi^2(2)$ = 7.79, P=.02

6.4 Discussion

The aim of this study was to investigate whether monitoring of easily measurable stressors and symptoms could be used to early distinguish between AF and FOR. The major result was that the combination of the changes in subjective fatigue and readiness to train, measured on simple visual analogue scales (Piacentini and Meeusen 2015), classified 78% of the subjects correctly as AF or FOR after only three days of cycling.

Classification AF versus FOR

The main reason to distinguish between AF and FOR in this study was that it has been shown in previous work that the supercompensation effect is smaller in FOR than in AF (Aubry et al. 2014; Aubry et al. 2015). In accordance with those studies the SWC in performance was used for classification of AF/

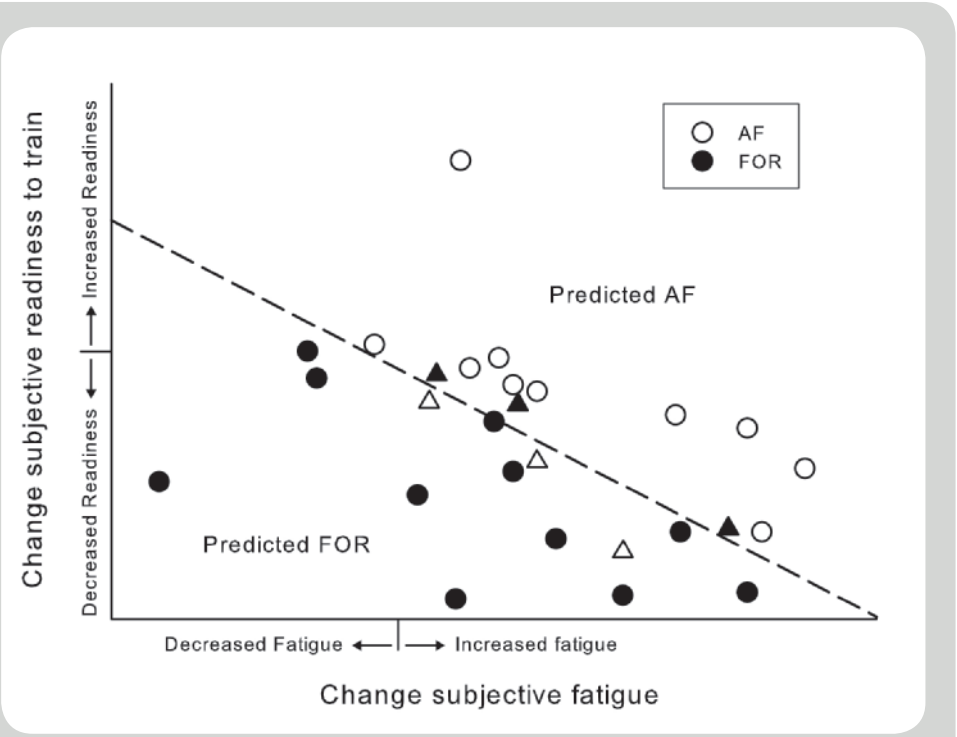


Figure 6.5. Change in subjective fatigue and change in subjective readiness to train for acute fatigued (AF) and functional overreached (FOR) subjects. The dashed line represents the demarcation line which follows from the β-values from the logistic regression. Subjects left/down from the demarcation line are predicted FOR, right/upper predicted as AF. Circles are correctly classified subjects, triangles incorrect classifications. Two subjects with multiple missing data were excluded from logistic regression analysis, resulting in 27 data points.

FOR in the current investigation. The SWC in our study (-0.5%) was of the same magnitude as in the previous work (-0.6%) (Aubry et al. 2014; Aubry et al. 2015). Using this method, 15 subjects were classified as FOR and 14 as AF. It is hard to compare these numbers to other investigations because the exercise volume in our study was very high. To illustrate, the total cycling time was twice as high as the total race time of professional cyclists in an 8-day race (Rodríguez-Marroyo et al. 2009). The increase of external training load in our study (approximately 900%) was much higher than the generally

applied increase in training load by 30%-100% (Aubry et al. 2014; Le Meur, Hausswirth, et al. 2013; Dupuy et al. 2014; Nederhof et al. 2007).
FOR precedes NFOR/OTS, the part of the overtraining continuum in which more severe and persistent symptoms occur. So it is possible that some of the FOR athletes in this study developed into the NFOR status. NFOR athletes typically need weeks to months for recovery of performance (Meeusen et al. 2006; Meeusen et al. 2013). On group level the peak power output was probably still lower (P=.12) in FOR compared to AF in the follow-up measurements 5 weeks after TFL (Figure 6.3). Yet, this measurement was performed near the end of the Dutch amateur road cycling season. Some subjects did not cycle anymore after the TFL while others continued training. Therefore the performance decrement may not be solely related to the effort of TFL. For that reason it was impossible to categorise subjects as FOR or NFOR based on follow-up performance decrement. Possibly endocrine disturbances (Meeusen et al. 2004) can be used in future research to further categorise FOR/NFOR subjects.

Predictors of FOR

Fatigue and readiness to train were the best predictors of FOR. Increased perceived fatigue has often been described in FOR athletes (Le Meur, Pichon, et al. 2013; Dupuy et al. 2014). However, to our knowledge, a change in fatigue has not been used before to predict FOR. In our study fatigue was not different between AF and FOR after any of the stages (Figure 6.4 B). Also the sensitivity (67%) and specificity (36%) were not high when fatigue was the sole predictor of FOR. Yet, the predictive power was substantially improved when combined with changes in readiness to train. Readiness to train is not often mentioned with regard to FOR. Urhausen et al. (1998) showed a significant alteration of readiness for effort in 15 cases of overtraining compared to non-overtraining periods in the same athletes. The overtraining status was not specified because the work was published before the FOR/

NFOR/OTS consensus statement (Meeusen et al. 2006).
Figure 6.5 shows the individual data points for changes in fatigue and readiness to train. This figure illustrates that a subject who felt less ready for the next stage, while equally fatigued, has a higher chance to become FOR. The combination of change in fatigue and readiness predicted 78% of the subjects in the correct class. In the study by Urhausen et al. (1998) 87% of the cases of overtraining could be recognised by a combination of decreased maximal lactate concentration, maximal heart rate, time to exhaustion and a self-condition scale (including readiness for effort). Le Meur and Haussiwirth et al. (2013) distinguished between athletes who continued their normal training and FOR. They were able to classify 89.5% of their subjects correct based on heart rate and lactate concentration during a maximal incremental running test. Subjects who responded well to the overload period (i.e. AF) were not included in this discriminant analysis. The sensitivity (79%) in our study was somewhat lower, but 3 differences between the studies should be taken into account: 1) we distinguished between AF and FOR, while Le Meur and Hausswirth et al. (2013) distinguished between subjects that continued their normal training and FOR. Based on their figures (Le Meur, Hausswirth, et al. 2013), the sensitivity would have been considerably lower when a distinction was made between AF and FOR, 2) we classified subjects after 3 days instead of 3 weeks of exercise, and 3) we used 2 visual analogue scales instead of a maximal incremental test to predict FOR. These are big advantages for early detection of FOR in training practice.

sRPE, resting heart rate and POMS

In contrast to fatigue and readiness to train, other monitoring tools such as the sRPE method, resting heart rate and POMS (except fatigue) seemed less useful to distinguish between AF and FOR in this study.
sRPE fluctuated per stage whereas fatigue increased progressively during the TFL (Figure 6.4). Perhaps this indicates that the sRPE represents only the

effort of the specific stage, while subjects integrated accumulated fatigue and recovery in the fatigue score. We speculate that the cumulative fatigue is more relevant than the day-to-day perceived exertion with regards to the development of FOR.

In a meta-analysis it was shown that resting heart rate was increased after intensified training shorter than two weeks (Bosquet et al. 2008). In our study heart rate also increased during the TFL, but without differences between the groups. Many factors influence the resting heart rate, such as body position and autonomic activity (Buchheit 2014). Conditions were difficult to control in this observational field study, in which subjects measured their resting heart rate in their tent at a campsite. This may have resulted in a signal-to-noise ratio too low to pick up differences between the groups.

In this study changes in all POMS subscales (except depression) were observed, but without differences between AF and FOR. The most likely explanation could be in the nature of the TFL, in which many emotions interfered and mood disturbances probably had other causes than FOR. For example, anger increased halfway through the event, perhaps due the intensive contact between team members. Also, tension was highest at the start of the TFL, perhaps because the riders did not exactly know what to expect or if they could make it to the finish. Micklewright et al. (2009) showed that performance expectations influence post-race mood. This suggests that monitoring mood might not be suitable in exceptional races such as TFL.

Practical application

This study shows that monitoring both the changes in fatigue and readiness to train, using simple visual analogue scales, can be used to early distinguish between FOR and AF. Hence, we encourage athlete support staff to not only monitor fatigue, but also the subjective integrated mental and physical readiness to perform of their athletes. Monitoring of these factors is easily applied in training practice, it is not demanding for athletes and can be

implemented with minimal costs.

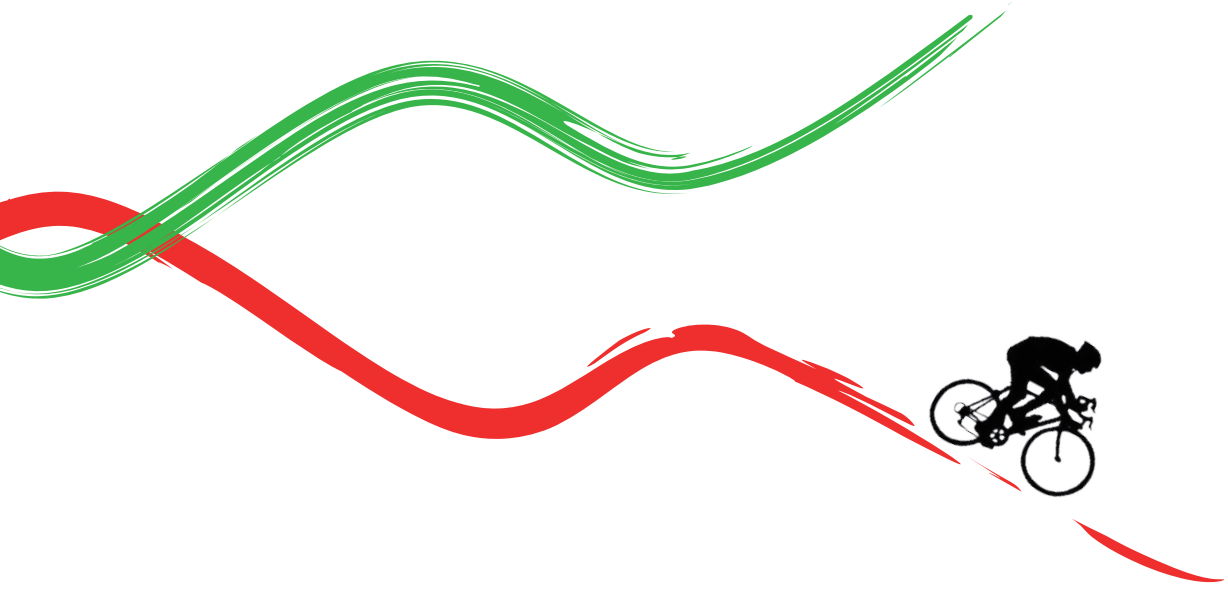
This study was conducted in a specific natural model, involving recreational athletes experiencing an enormous increase in training load. Further research should focus on application to high level and elite athletes in their normal training cycles. Also, prolonged monitoring of changes in fatigue and readiness is advised.

6.5 Conclusion

This study shows that monitoring of easy measurable symptoms can be used to identify FOR after only 3 days of cycling. Monitoring changes in fatigue and readiness to train, using simple visual analogue scales, had the highest predictive power to identify FOR.

Chapter 7

General discussion



Athletes sometimes deliberately increase their training load with the goal to increase their performance capacity. However, insufficient recovery during such periods of intensified training will result in functional overreaching (FOR). FOR athletes might show a smaller or even absent performance gain after a period of rest compared to AF athletes (Aubry et al. 2014), and suffer from reduced sleep quality and increased risk of infections (Hausswirth et al. 2014). Moreover, a long-lasting imbalance between (exercise) stress and recovery may result in the more severe stages of the overtraining spectrum, i.e. NFOR or OTS. Therefore, the aim of this thesis was to identify parameters that early distinguish between amateur cyclists who underperformed (FOR) and those who were able to maintain performance (AF) after a period of intensified training. This thesis covered some of the most promising parameters, namely hormonal levels, heart rate, choice reaction time, and monitoring of stressors and symptoms of overreaching (Meeusen et al. 2013).

7.1 Validity of the model

The Tour for Life (TFL) was adopted as a natural occurring experimental model to investigate the overtraining spectrum throughout this PhD thesis. The TFL model involves a large increase in exercise volume, applied to a population with a various exercise capacity. Therefore, it was expected that this model would enable us to study a heterogeneous response to intensified training, i.e. athletes who underperformed (FOR) and maintained performance (AF) as a result of the TFL. Out of the 400 contestants of the TFL, 30 subjects were recruited for the research project. The recruited cyclists were of performance level 1 (4%), 2 (57%), 3 (25%), and 4 (14%), according to the $\dot{V}O_2$max-based guidelines to describe subjects in sport-science research (De Pauw et al. 2013; Decroix, De Pauw, et al. 2016). The exercise volume during the TFL was on average approximately 9 fold compared to the preparation period. This resulted in an average (AF+FOR) post versus pre TFL decrease in power output of 2.7%. To give an indication, a 2.7% decrease in power output in

the Rio 2016 Olympic men's road cycling time trial equals the estimated difference between a place at the podium and rank 6 (de Koning, Bobbert, and Foster 1999). Of course, for various reasons this is a very rough comparison (e.g. endurance performance versus peak power output, and assumption of constant power output), but it gives an impression of the magnitude of the change in performance. In line with our expectations, a heterogeneous change in performance was observed, ranging between a 15% decrease and a 6% increase. Based on the criterion of the smallest worthwhile change in performance, 15 subjects were classified as FOR and 14 as AF (1 subject was excluded). Thus, it can be concluded that the TFL model was a valid model to investigate which of the most promising parameters can be used to distinguish between FOR and AF athletes.

The research model in this thesis holds two assumptions that may be a point of debate. Firstly, the single criterion applied to distinguish between AF and FOR was a decrease in performance that was larger than the smallest worthwhile change. Some other studies classified subjects based on other criteria, such as (combinations of) ratings of fatigue, mood disturbances, increase or decrease of heart rate, or decreased maximal blood lactate (Brink, Visscher, Schmikli, et al. 2012; Dupuy et al. 2014; Nederhof et al. 2007; Schmikli et al. 2012). However, a decrease in performance is the main characteristic of the overtraining spectrum, and seems therefore a reasonable classification criterion. The smallest worthwhile change, as used in our study, has been applied by several other studies on overtraining (Aubry et al. 2014; Aubry et al. 2015; Le Meur et al. 2014; Decroix, Piacentini, et al. 2016). Moreover, the threshold of the performance change in our study model (coefficient of variation: 1.6%, smallest worthwhile change: 0.5%) was similar to previous studies (Aubry et al. 2014; Aubry et al. 2015). In conclusion, the method applied to distinguish between AF and FOR in this thesis adds to the continuity and uniformity in the recent literature on FOR, and enhances comparability between results. The second assumption was that the observed changes after the TFL were a result of overtraining rather than detraining. A review on the

effects of training cessation (in athletes without overtraining) showed that hormonal levels did not change after 5 days of detraining, and that exercise heart rate was similar or even increased (as opposed to the decrease in our study) (Mujika and Padilla 2000). Thus, it is likely that the observed changes post TFL (5 to 8 days after the event) were the result of overtraining. The origin of observed changes that were observed at follow-up (5 weeks after the event) is less clear. Some subjects reached their goal by finishing the TFL and did not continue training afterwards, while others maintained their pre TFL exercise volume. Hence, the observed effects 5 weeks after the TFL may be considered (partly) as detraining effects.

It is uncertain to what extent the results from this thesis can directly be translated to professional athletes. The first reason is that our amateur subjects were not as highly adapted to endurance exercise as professional athletes (e.g. $\dot{V}O_2$max in TFL subjects 51.8±6.3 $mL \cdot kg^{-1} \cdot min^{-1}$, professional cyclists 75 $mL \cdot kg^{-1} \cdot min^{-1}$ (Sanders et al. 2017)). This may affect the degree of disturbance in the investigated parameters. For example, it has recently been shown that OTS athletes showed the same hormonal responses to a stimulation test as sedentary controls, but reduced responses compared to healthy athletes (Cadegiani and Kater 2017c). It was therefore suggested that OTS athletes lost the positive hormonal adaptation that is acquired by healthy athletes. A second reason that limits translation of our results to professional athletes is the increase in exercise volume in our TFL model (9 fold compared to the preparation period). This increase is not expected in elite sports practice. The increase in exercise load in other studies is often 130%-200% compared to normal training, for the duration of 2-4 weeks (Dupuy et al. 2014; Halson et al. 2002; Le Meur, Hausswirth, et al. 2013; Meeusen et al. 2004; Rietjens et al. 2005). The TFL is a pressure-cooker-style model, with a very high relative exercise load in a short period. It is currently unknown whether this exerts the same mental and physical effects as a lower increase in training load spread out over a longer period.

7.2 The investigated parameters

In this thesis we focused on some of the most promising parameters to distinguish between AF and FOR. The findings on hormonal levels, heart rate, choice reaction time, and monitoring of stressors and symptoms will be discussed in this section.

Hormonal system

It was hypothesized that in FOR athletes 1) hormonal levels during the TFL would change, 2) exercise-induced hormonal responses would be reduced after the event, most pronounced after the second exercise bout on a day, but 3) these changes would be absent or less noticeable in AF athletes. The findings in this thesis clearly demonstrated that disturbance of the hormonal system, as previously found in NFOR and OTS athletes (Barron et al. 1985; Meeusen et al. 2010; Cadegiani and Kater 2017c; Cadegiani and Kater 2017a), arise already in the early stages of the training-overtraining spectrum. Yet, the changes in the hormonal system were not different between AF and FOR athletes.

On total group level (AF+FOR), decreased resting ACTH, cortisol and PRL levels during the TFL, and reduced responses to exercise after the TFL were observed. Previous research on the overtraining spectrum showed that these changes in the endocrine system most likely originate at the level of the hypothalamus or pituitary (Cadegiani and Kater 2017c). Our data indicated that these brain structures were affected already after 4 days of cycling. The observed down-regulation of the hormonal system can currently not be explained. It has been suggested to be a loss of the positive adaptation acquired by healthy athletes, possibly explaining the reduced performance capacity that characterizes overtraining (Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). An alternative, but not mutually exclusive, hypothesis is that this down-regulation may function as a protective mechanism against the harmful effects of a high allostatic load (Fries et al. 2005).

It has been argued that pharmacological tests are preferred over exercise stimulation tests, because pharmacological stimulation tests circumvent the potential effects of impaired signalling from the musculoskeletal and cardiovascular systems (Cadegiani and Kater 2017c; Cadegiani and Kater 2017a). Still, our findings showed reduced hormonal responses both in AF and FOR athletes. Thus, even when the duration and intensity of the post TFL exercise test was equal or increased compared to pre TFL, lower hormonal responses were shown. This suggests a limited role of the signalling pathways, and justifies the use of exercise test to study responsiveness of the hormonal system.

Resting GH levels increased during the TFL. A strong negative association was found between the change in GH during the TFL and the pre versus post TFL change in performance (r=-.63 and r=-.68 for morning and afternoon GH levels, respectively). GH has an important role in metabolism and protein synthesis. Its main function in periods of energy deficit is to increase lipid mobilization and oxidation (Møller and Jørgensen 2009). Due to the nature of the TFL model, the energy balance of the subjects was not controlled. Yet, the TFL elicits high metabolic demands, illustrated by cycling on average 495 minutes per day with a session Rating of Perceived Exertion of 6.9±1.2 [0-10 scale] for 8 consecutive days. In addition, the subjects were inexperienced with such high energetic demands and corresponding nutritional needs. It is, therefore, tempting to speculate that the elevated GH levels and decreased physical performance were related to an energy imbalance.

Heart rate

The findings presented in this thesis are in line with the hypothesis that (sub) maximal heart rate would be lower after the TFL, but that this decrease would not be associated with the change in performance.

A negative association with O_2pulse was observed. Moreover, (sub) maximal $\dot{V}O_2$ remained unchanged during the exercise tests. This indicated that a

reduced heart rate was compensated by either an increased stroke volume and/or an increased arteriovenous oxygen difference.
The cause of the reduced heart rate is unclear. The finding that heart rate at sub maximal exercise intensity was reduced, suggests that the reduced maximal heart rate is not (solely) the consequence of lower exercise duration and intensity. Lower (sub) maximal heart rate may be the result of (a combination of) reduced adrenergic catecholamine levels (Le Meur et al. 2014), increased parasympathetic activity (Le Meur, Pichon, et al. 2013; Bellenger et al. 2017), or decreased density and/or sensitivity of the adrenal receptors (Steinacker et al. 2004).
The observation that heart rate decreased after the TFL in both AF and FOR athletes implies that heart rate is of limited value to monitor intensified training. Additionally, the consequence of a reduced heart rate is that training schedules based on heart rate derived training zones result in underestimation of the internal training load. Thus, heart rate seems inadequate to prescribe and monitor intensified training.

Choice reaction time

The results in this thesis do not confirm our hypothesis that choice reaction time would be slower only in FOR but not in AF athletes. Instead, reaction time became faster in both groups of athletes.
Reaction time has been proposed in the most recent ECSS and ACSM joint consensus statement on overtraining as a promising tool to detect overtraining (Meeusen et al. 2013). However, until now, literature is scarce and ambiguous. It is unlikely that the inconsistent results were a consequence of different applied tests, since conflicting results have been found for the Finger-Precuing Task (Nederhof et al. 2007; Rietjens et al. 2005), Stroop test (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010), and simple reaction time test (Decroix, Piacentini, et al. 2016; Dupuy et al. 2010; Le Meur, Hausswirth, et al. 2013). Thus, the use of attention-based (e.g. simple reaction time) or

post-perceptual processing-based tests (e.g. Finger-Precuing Task) does not explain differences between studies. Neither do our and previous results support the threshold theory (Satz 1993), which suggests that reaction time is only reduced beyond a threshold of cognitive effort, as a valid explanation for ambivalent results. To conclude, it is unlikely that choice reaction time is a useful tool to distinguish between AF and FOR.

Monitoring of stressors and symptoms of overreaching

Among the investigated parameters in chapter 6, the combination of subjective fatigue and readiness to train had the highest power to predict AF and FOR in athletes after the TFL. By means of these subjective measures 78% of the subjects were correctly classified after only 3 days of cycling.

Fatigue is the direct result of the imbalance between (exercise-induced) stress and recovery. The perception of readiness to perform is a critical determinant of recovery, according to the recent consensus statement on recovery and performance in sport (Kellmann et al. 2018). Thus, subjective fatigue and readiness to train arguably represent the total sum of stressors and recovery, and directly relate to the ability to perform. In contrast, the other investigated parameters measure only a specific stressor or symptom. Yet, these stressors and symptoms might differ between individuals and are situational dependent. For example, the POMS assesses mood disturbance, which might be, but not necessarily is, a result of imbalanced stress and recovery (i.e. overtraining). Also, sleep quality, muscle soreness, and resting heart rate are specific symptoms, which might differ between and within athletes under identical external load. The session Rating of Perceived Exertion represents a measure of intensity and load (Bourdon et al. 2017; Kellmann et al. 2018), but does not assess recovery. This is illustrated by our data (Figure 6.4), which showed that sRPE varies per stage, whereas ratings of fatigue showed an accumulating pattern. To conclude, subjective fatigue and readiness to train seem to represent the total stress-recovery balance. Assessment of these two factors on a visual

analogue scale was the most powerful method to distinguish between AF and FOR athletes.

How to distinguish between AF and FOR

From an integrative perspective on the results that were presented in this thesis, it becomes clear that monitoring of the total stress-recovery balance (Figure 1.1) is the best method to early distinguish between athletes that underperform and athletes that maintain their performance level after an 8-day non-competitive amateur cycling event. This finding is in line with a recent review on load monitoring, that concluded that regular assessment of self-report measures surpasses any physiological measure (Bourdon et al. 2017). The very basic subjective ratings of fatigue and readiness to train reflect the stress-recovery balance. This balance integrates exercise and non-training related stressors and recovery, and is fundamental to the various physical and mental symptoms of overtraining. Besides this strong theoretical basis, frequent monitoring of fatigue and readiness to train is inexpensive, it is easily applicable in training practice, and it is hardly demanding for athletes. Hence, it fulfils important requirements for training monitoring tools, and thus has the potential for good compliance and valuable results (Nederhof et al. 2006). The studies in this thesis did not identify a single performance-limiting factor in amateur cyclists after an 8-day non-competitive event. This means that the limiting factor was not amongst the investigated parameters in this thesis, and/or the performance limiting factor differs between subjects, and/or performance is reduced by a combination of factors. Most likely, overtraining originates from an interplay between several physiological systems (Meeusen et al. 2013). Hence, it might be hard, or even theoretically impossible, to pinpoint a single physiological determinant of the change in performance in FOR athletes. Besides the various possible (combinations of) physiological determinants, motivation may play a role in the change in performance. Changed mood and vigour have frequently been reported amongst athletes

in the overtraining spectrum (Saw et al. 2017; Meeusen et al. 2013). Thus, a potential change in the mental capacity to perform cannot be excluded as a (partial) explanation of decreased performance after the TFL.
Although the examined physiological measures in this thesis seem inadequate to distinguish between AF and FOR, the studies on the hormonal system and heart rate provided insight in (the development of) the overtraining spectrum. For example, decreased resting ACTH, cortisol and PRL levels during the TFL showed that the hormonal system is affected already in the early stages of the spectrum (AF/FOR). This was also illustrated by reduced responsiveness to exercise after the TFL. Moreover, heart rate during (sub) maximal exercise was shown to decrease, but, at least in the AF/FOR stage of the spectrum, this appears to be compensated by other physiological mechanisms.

7.3 Future directions for research

The results of this thesis showed that monitoring of the complete stress-recovery balance, rather than specific stressors or symptoms, was most powerful to early distinguish between amateur cyclist that showed decreased performance and those who maintained their performance level. In this thesis, the stress-recovery balance was assessed using subjective fatigue and readiness to train, rated on visual analogue scales (VAS). Yet, specific instruments have been developed to examine this (im)balance. An example is the RESTQ-Sport, a 76-item questionnaire on the frequency of stressors and recovery-associated activities of the last 3 days and nights (Kellmann and Kallus 2001). Recently, shorter versions such as the Acute Recovery and Stress Scale and the Short Recovery and Stress Scale have been developed to meet the demands for quick and easily implementable tools (Nässi et al. 2017). Still, these tools require more time and effort from an athlete than 2 simple VAS items, which may have consequences for the compliance rate (Halson 2014). The additional sensitivity of these tools above the fatigue/readiness VAS items is currently unknown. Ideally, this is investigated in an intention-to-treat design that

considers both the compliance and the sensitivity to distinguish between AF and FOR.

Other topics of this thesis hold their own directions for future research, mainly focused on enlighten the underlying mechanisms of the physiological disturbances that were observed. Firstly, it was shown that (sub) maximal heart rate decreased after the TFL. The fundamental physiological mechanism, however, is currently unknown. Future research might investigate the (partial) role of changed adrenergic catecholamine levels (Le Meur et al. 2014), increased parasympathetic activity (Bellenger et al. 2017; Le Meur, Pichon, et al. 2013), and decreased density and/or sensitivity of the adrenal receptors (Steinacker et al. 2004). In addition, it was shown that the decrease in heart rate was nullified by an increased stroke volume or arteriovenous oxygen difference. The physiological basis of the counteracting mechanism might be elucidated in future research. Secondly, reduced hormonal responses to exercise have been shown in this thesis. Recent research provided evidence for adaptations of the hypothalamus or pituitary (Cadegiani and Kater 2017c). Reduced hormonal levels after a period of stress are suggested to result from 1) reduced biosynthesis or depletion of CRH or ACTH, 2) increased negative feedback sensitivity to cortisol, or 3) down-regulation of receptors in the hypothalamus or pituitary (Fries et al. 2005). Future research may focus on these possible causes of the down-regulation of the hormonal system, and thereby gaining deeper insight into the physiological adaptations associated with the overtraining spectrum.

Overtraining is regarded as a multifactorial phenomenon with links to numerous fields in medicine and exercise science. This thesis covered only some of the most promising parameters. Still, we recently collaborated with experts in (sports) cardiology and immunology, and used the TFL model to investigate emerging topics in these fields. For example, it has been shown that excessive exercise, such as running a marathon, can result in increased levels of markers for cardiac damage (Eijsvogels et al. 2015). To investigate whether markers for cardiac damage increased during the TFL, blood samples

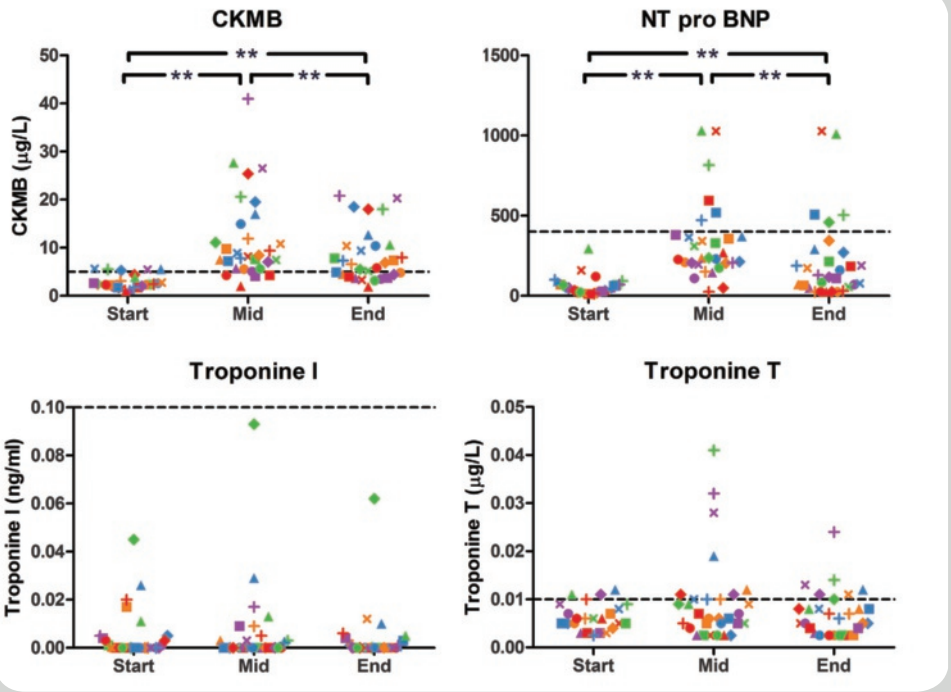


Figure 7.1. CK-Mb, Nt-proBNP, Troponine-I and Troponine-T levels at the start, halfway and end of the Tour for Life. Colour and symbol combinations respresent the individual subjects. The horizontal dashed line represents the thresholds generally used to rule in cardiac pathology. ** P<.001

drawn at the start, halfway, and end of the TFL were analysed for CK-Mb, Nt-proBNP, and troponine I and T. Preliminary results showed that CK-Mb and Nt-proBNP levels were significantly (P<.001) higher halfway and at the end compared to the start of the TFL (Figure 7.1). Moreover, large individual differences were observed, with some subjects showing levels above the thresholds that are generally used to rule in cardiac pathology. Previous research has shown that these markers may be elevated due to non-cardiac causes, such as renal insufficiency or muscular dystrophy (Diris et al. 2004; Hoogerwaard et al. 2001). Future studies may combine these cardiac markers with functional imaging to elucidate whether these elevated levels observed

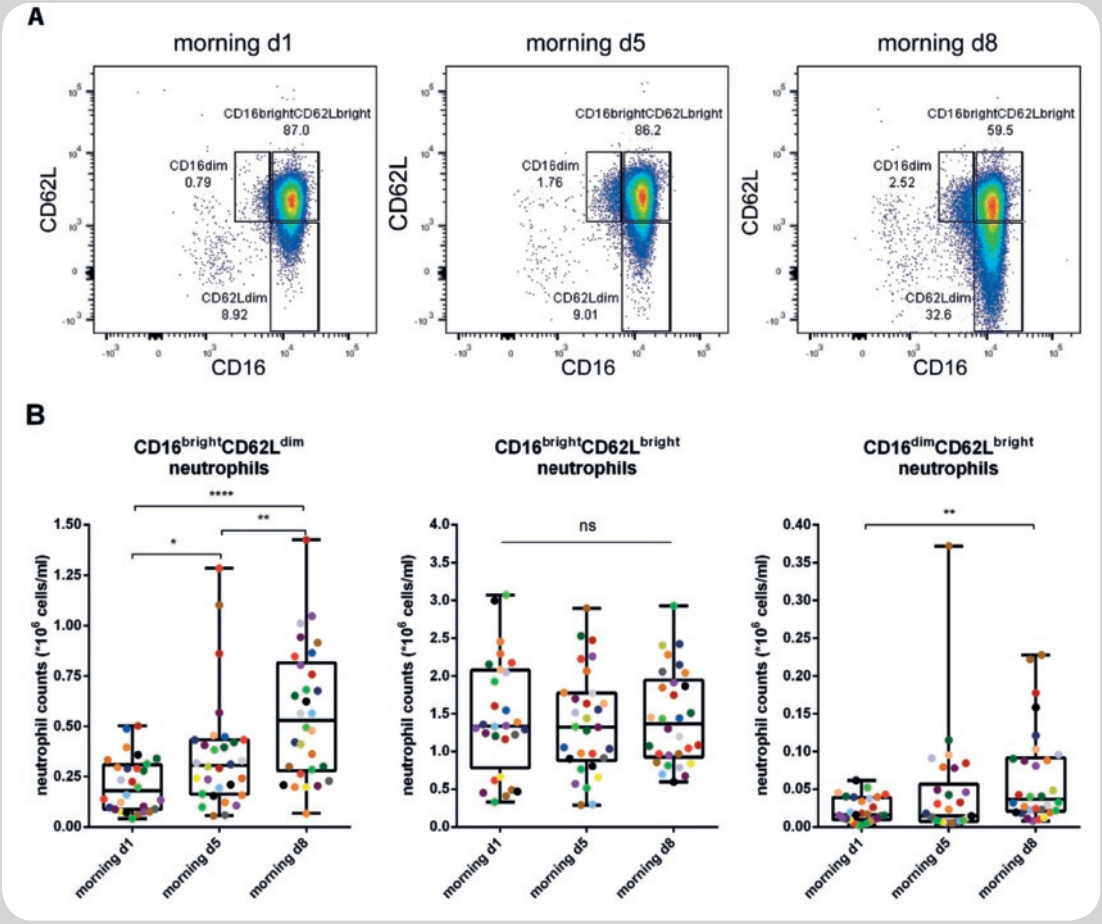


Figure 7.2. CD16 and CD62L neutrophil subsets at the start, halfway, and end of the Tour for Life. (**A**) Representative FACS plot of CD16 versus CD62L expression of the total neutrophil population of one individual at the 3 morning time points during the TFL. (**B**) graphs of the three CD16CD62L neutrophil subset counts per individual (n=28) at 3 morning time points during the TFL (d1: day 1 (start), d5: day 5 (halfway), d8: day 8 (end)). Colours visualize the individual changes over time.

ns: not significant; * P<.05; ** P<.005; **** P<.0001.

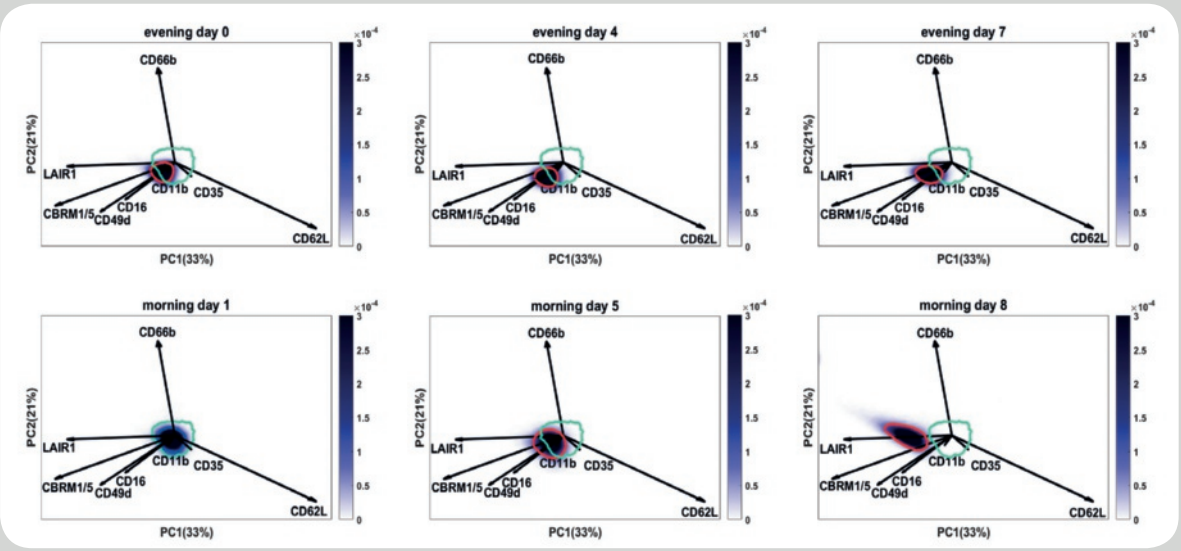


Figure 7.3. Multidimensional FLOOD analysis shows results found by conventional gating analyses in one biplot. FLOOD output: neutrophil deep phenotyping results at evening day 0, morning day 1, evening day 4, morning day 5, evening day 7 and morning day 8 in the multivariate response space. Results of six time point of one representative subject are shown here. Cells are depicted in a blue density gradient. The length of the vector of each marker correlates to the relative importance of that marker for the description of the multivariate space. The correlations between the different markers is defined by the angle between the vectors. Vectors pointing in the same direction are positively correlated; vectors in an angle 90^0 are not correlated with each other; vectors positioned in an angle of 180^0 are negatively correlated. The cyan circle depicts the region of 80% of the cells of all the control samples (day 1).The dark blue benchmark in the morning day 1 plot encompasses 80% of the cells of this specific sample. The neutrophils of this control sample fall within the cyan control benchmark. In the graphs of the other time points the red benchmark encompasses 80% of the cells of this individual. The cells shifted in the multivariate space towards CBRM1/5, LAIR-1, CD49d and CD16. Overnight from evening day 4 to morning day 5 the shift was maintained, but part of the cells still overlapped with the control benchmark. A total shift of the cells was seen at morning day 8, in the opposite direction of CD62L and more towards LAIR-1, CBRM1/5 and CD49d. Also a larger spread of the cells was found in the negative direction of CD62L.

in athletes after intensified training indicate cardiac damage, or arise from excessive peripheral muscle damage.

A second ongoing collaboration is with the field of exercise immunology. The overtraining spectrum has often been associated with an increased incidence of minor illnesses, such as upper respiratory tract infections (URTI), likely caused by an impaired immune response (MacKinnon 2000). It has been suggested that neutrophils have an important role in the immunological response to exercise (Robson et al. 1999). The TFL model was used to get more insight into the potential role of neutrophils in the impaired immune response during periods of intensified training. The results showed a 1.26 (95% CI [1.01, 1.51]) fold increase in the morning total neutrophil counts at the end compared to the start of the TFL. Moreover, flow cytometry measurements revealed $CD16_{bright}CD62L_{dim}$ and $CD16_{dim}CD62L_{bright}$ neutrophil subsets, which appeared in addition to the homogeneous $CD16_{bright}CD62L_{bright}$ neutrophil population that was observed under homeostasis. (Figure 7.2). The $CD16_{dim}CD62L_{bright}$ cells are less mature, and are likely recruited from the bone marrow. The other mobilized subset consists of $CD16_{bright}CD62L_{dim}$ neutrophils, that exhibit a more activated and immunosuppressive phenotype. In acute inflammation $CD16_{bright}CD62L_{dim}$ and $CD16_{dim}CD62L_{bright}$ neutrophil subsets also appear in the peripheral blood. However, after a single LPS challenge, an experimental model for acute inflammation, the blood neutrophil compartment completely normalized overnight (Kamp et al. 2013). This is in contrast to our findings during the TFL, during which these neutrophil subsets were found in resting morning blood samples. Apparently, repeated endurance exercise during the TFL evoked persistent signals leading to chronic mobilization of the different neutrophil subsets. By means of a recent developed tool for multidimensional analysis (FLOOD) we were able to study complex differences in neutrophil phenotypes in more detail (Jansen et al. 2016). This change was characterized by decreased expression of CD11b and CD62L, and increased expression of LAIR-1, CBRM1/5, and CD49d (Figure 7.3). The exercise-induced upregulation of LAIR-1, CBRM1/5,

and CD49d did not recover overnight. In fact, the shift was maintained at morning day 5, and even more pronounced at morning day 8 than directly post-exercise at day 7. In conclusion, a build-up in changes in the neutrophil compartment was identified in the blood at day 5 and 8 of the TFL with a complex change in marker expression. Future research may shed more light on the role of neutrophils in impaired immune function and the increased risk for URTI during intensified training.

7.4 Practical application

Based on the results of this thesis, professionals working with athletes are encouraged to monitor the balance between (exercise-induced) stress and recovery. In this thesis, the stress-recovery balance was assessed by means of subjective ratings of fatigue and readiness to train. Yet, practitioners should adapt, rather than fully adopt, scientific findings to their athletes (Bourdon et al. 2017). This means that the method to assess the stress-recovery balance must be selected based on the preferences and convictions of the athletes and coaches, available resources, and frequency of monitoring. For example, the use of 2 VAS items requires minimal time and effort, and can easily be interpreted. Yet, the downside of this method is that it does not inform about the stressor(s) or recovery method(s) that cause an imbalance. In contrast, the RESTQ-Sport requires more effort of athletes, and thus holds a risk of lower compliance. Yet, it has the advantage that it includes items on current stressors and recovery-associated activities. Therefore, recovery strategies can be tailored to the nature of the present stressor(s) (Kellmann et al. 2018). Practitioners might consider using a concise tool for daily assessment of stress-recovery to quickly screen for athletes at risk, combined with more detailed examination of vulnerable subjects. It should be noted that exclusively relying on subjective measures holds the risk of being misled by athletes in an attempt to influence training (Bourdon et al. 2017). This threat to effective monitoring can be reduced by practitioners by informing and educating their

athletes. Still, a combination of subjective and objective measures might be considered. Regardless the method that is selected as the most suitable to the specific sports setting, practitioners are advised to use individualized thresholds for smallest worthwhile changes (Halson 2014).
A secondary practical advice is derived from our study on heart rate. Heart rate is often used to prescribe and monitor (intensified) training. Yet, it was shown that heart rate during exercise decreased after the TFL, but without differences between AF and FOR athletes. Thus, (sub) maximal heart rate measures seem inadequate to distinguish between a stress-recovery imbalance (FOR) and effective training (AF). Moreover, because of the generally observed decrease in heart rate, relying on heart rate to prescribe training(zones) will result in an unintentional high internal training load. To summarize, the results of this thesis advise against the use of heart rate to prescribe and monitor intensified training.

7.5 Conclusion

The aim of this thesis was to identify parameters that early distinguish between amateur cyclists who underperformed (FOR) and those who were able to maintain performance (AF) after a period of intensified training. Down-regulation of the heart rate during exercise and the hormonal system were observed already in this stage of the overtraining spectrum. However, changes in these determinants of physical performance were not different between AF and FOR. Instead, monitoring of the stress-recovery balance (which reflects the underlying process of the overtraining spectrum) by means of subjective ratings of fatigue and readiness to train was shown to be the most powerful tool to distinguish between AF and FOR. Professionals working in sports are, therefore, encouraged to incorporate frequent monitoring of the stress-recovery balance in supervision of their athletes. It is advised to adapt the methodology so that it best fits the situation and desires of the athletes and coaches.

References

Achten, J, and Jeukendrup, AE. 2003. "Heart Rate Monitoring: Applications and Limitations." *Sports Med* 33 (7): 517–38.

Adam, JJ, Paas, FG, Teeken, JC, van Loon, EM, van Boxtel, MP, Houx, PJ, and Jolles, J. 1998. "Effects of Age on Performance in a Finger-Precuing Task." *J Exp Psychol Hum Percept Perform* 24 (3): 870–83.

Armstrong, LE, and VanHeest, JL. 2002. "The Unknown Mechanism of the Overtraining Syndrome: Clues from Depression and Psychoneuroimmunology." *Sports Med* 32 (3): 185–209.

Aubry, A, Hausswirth, C, Louis, J, Coutts, AJ, Buchheit, M, and Le Meur, Y. 2015. "The Development of Functional Overreaching Is Associated with a Faster Heart Rate Recovery in Endurance Athletes." *PlosOne* 10 (10): e0139754.

Aubry, A, Hausswirth, C, Louis, J, Coutts, AJ, and Le Meur, Y. 2014. "Functional Overreaching: The Key to Peak Performance during the Taper?" *Med Sci Sports Exerc* 46 (9): 1769–77.

Barron, JL, Noakes, TD, Levy, W, Smith, C, and Millar, RP. 1985. "Hypothalamic Dysfunction in Overtrained Athletes." *J Clin Endocrinol Metab* 60 (4): 803–6.

Beaver, WL, Wasserman, K, and Whipp, BJ. 1986. "A New Method for Detecting Anaerobic Threshold by Gas Exchange." *J Appl Physiol* 60 (6): 2020–27.

Belanger, HG, Curtiss, G, Demery, JA, Lebowitz, BK, and Vanderploeg, RD. 2005. "Factors Moderating Neuropsychological Outcomes Following

Mild Traumatic Brain Injury: A Meta-Analysis." *J Int Neuropsychol Soc* 11 (3): 215–27.

Bellenger, CR, Thomson, RL, Robertson, EY, Davison, K, Nelson, MJ, Karavirta, L, and Buckley, JD. 2017. "The Effect of Functional Overreaching on Parameters of Autonomic Heart Rate Regulation." *Eur J Appl Physiol* 117 (3): 541–50.

Ben-Jonathan, N, Hugo, ER, Brandebourg, TD, and LaPensee, CR. 2006. "Focus on Prolactin as a Metabolic Hormone." *Trends Endocrinol Metab* 17 (3): 110–16.

Bornstein, SR, Engeland, WC, Ehrhart-Bornstein, M, and Herman, JP. 2008. "Dissociation of ACTH and Glucocorticoids." *Trends Endocrinol Metab* 19 (5): 175–80.

Bosquet, L, Merkari, S, Arvisais, D, and Aubert, AE. 2008. "Is Heart Rate a Convenient Tool to Monitor Over-Reaching? A Systematic Review of the Literature." *Br J Sports Med* 42 (9): 709–14.

Bourdon, PC, Cardinale, M, Murray, A, Gastin, P, Kellmann, M, Varley, MC, Gabbett, TJ, et al. 2017. "Monitoring Athlete Training Loads: Consensus Statement." *Int J Sports Physiol Perform 12 (Suppl 2):* 161–70.

Brink, MS, Visscher, C, Coutts, AJ, and Lemmink, KAPM. 2012. "Changes in Perceived Stress and Recovery in Overreached Young Elite Soccer Players." *Scan J Med Sci Sports* 22 (2): 285–92.

Brink, MS, Visscher, C, Schmikli, SL, Nederhof, E, and Lemmink, KAPM. 2012. "Is an Elevated Submaximal Heart Rate Associated with Psychomotor Slowness in Young Elite Soccer Players?" *Eur J Sports Sci* 13 (2): 207–14.

Brink, MS, Visscher, C, Arends, S, Zwerver, J, Post, WJ, and Lemmink, KAPM. 2010. "Monitoring Stress and Recovery: New Insights for the Prevention of Injuries and Illnesses in Elite Youth Soccer Players." *Br J*

Sports Med 44 (11): 809–15.

Bruce, RA, Kusumi, F, Culver, BH, and Butler, J. 1975. "Cardiac Limitation to Maximal Oxygen Transport and Changes in Components after Jogging across the US." *J Appl Physiol* 39 (6): 958–64.

Buchheit, M. 2014. "Monitoring Training Status with HR Measures: Do All Roads Lead to Rome?" *Front Physiol* 5 (73): 1–19.

Cadegiani, FA, and Kater, CE. 2017a. "Growth Hormone (GH) and Prolactin Responses to a Non-Exercise Stress Test in Athletes with Overtraining Syndrome: Results from the Endocrine and Metabolic Responses on Overtraining Syndrome (EROS) - EROS-STRESS." *J Sci Med Sport* Epub ahead of print.

Cadegiani, FA, and Kater, CE. 2017b. "Hormonal Aspects of Overtraining Syndrome: A Systematic Review." *BMC Sports Sci Med Rehabil* 9 (14).

Cadegiani, FA, and Kater, CE. 2017c. "Hypothalamic-Pituitary-Adrenal (HPA) Axis Functioning in Overtraining Syndrome: Findings from Endocrine and Metabolic Responses on Overtraining Syndrome (EROS)—EROS-HPA Axis." *Sports Med Open* 3 (45).

Chang, YK, Labban, JD, Gapin, JI, and Etnier, JL. 2012. "The Effects of Acute Exercise on Cognitive Performance: A Meta-Analysis." *Brain Res* 1453 (250): 87–101.

Christen, J, Foster, C, Porcari, JP, and Mikat, RP. 2016. "Temporal Robustness of the Session RPE." *Int J Sports Physiol Perform* 11 (8): 1088-93.

Chrousos, GP. 1998. "Stressors, Stress, and Neuroendocrine Integration of the Adaptive Response. The 1997 Hans Selye Memorial Lecture." *Ann N Y Acad Sci* 851 (1): 311–35.

Comotto, S, Bottoni, A, Moci, E, and Piacentini, MF. 2015. "Analysis of

Session-RPE and Profile of Mood States during a Triathlon Training Camp." *J Sports Med Phys Fitness* 55 (4): 361–67.

Cousineau, D. 2005. "Confidence Intervals in Within-Subject Designs: A Simpler Solution to Loftus and Masson's Method." *Tutorials in Quantitative Methods for Psychology* 1 (1): 42–45.

Coutts, AJ, Reaburn, P, Piva, TJ, and Rowsell, GJ. 2007. "Monitoring for Overreaching in Rugby League Players." *Eur J Appl Physiol* 99 (3): 313–24.

Coutts, AJ, Wallace, LK, and Slattery, KM. 2007. "Monitoring Changes in Performance, Physiology, Biochemistry, and Psychology during Overreaching and Recovery in Triathletes." *Int J Sports Med* 28 (2): 25–34.

Darwin, C. 1859. *On The Origin of Species by Means of Natural Selection, or Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

De Geus, EJC, Willemsen, GHM, Klaver, CHAM, and Van Doornen, LJP. 1995. "Ambulatory Measurement of Respiratory Sinus Arrhythmia and Respiration Rate." *Biol Psychol* 41 (3): 205–27.

de Koning, JJ, Bobbert, MF, and Foster, C. 1999. "Determination of Optimal Pacing Strategy in Track Cycling with an Energy Flow Model." *J Sci Med Sport* 2 (3): 266–77.

De Pauw, K, Roelands, B, Cheung, SS, de Geus, B, Rietjens, G, and Meeusen, R. 2013. "Guidelines to Classify Subject Groups in Sport-Science Research." *Int J Sports Physiol Perform* 8 (2): 111–22.

Deary, IJ, Liewald, D, and Nissan, J. 2011. "A Free, Easy-to-Use, Computer-

Based Simple and Four-Choice Reaction Time Programme: The Deary-Liewald Reaction Time Task." *Behav Res Methods* 43 (1): 258–68.

Decroix, L, De Pauw, K, Foster, C, and Meeusen, R. 2016. "Guidelines to Classify Female Subject Groups in Sport Science Research." *Int J Sports Physiol Perform* 11 (2): 204–13.

Decroix, L, Lamberts, RP, and Meeusen, R. 2018. "Can the Lamberts Submaximal Cycle Test Reflect Overreaching in Professional Cyclists?" *Int J Sports Physiol Perform* 13 (1): 23–28.

Decroix, L, Piacentini, MF, Rietjens, G, and Meeusen, R. 2016. "Monitoring Physical and Cognitive Overload during a Training Camp in Professional Female Cyclists." *Int J Sports Physiol Perform* 11 (7): 933–39.

Diris, JHC, Hackeng, CM, Kooman, JP, Pinto, YM, Hermens, WT, and Van Dieijen-Visser, MP. 2004. "Impaired Renal Clearance Explains Elevated Troponin T Fragments in Hemodialysis Patients." *Circulation* 109 (1): 23–25.

Duclos, M, Guinot, M, and Le Bouc, Y. 2007. "Cortisol and GH: Odd and Controversial Ideas." *Appl Physiol Nutr Metab* 32 (5): 895–903.

Dupuy, O, Lussier, M, Fraser, S, Bherer, L, Audiffren, M, and Bosquet, L. 2014. "Effect of Overreaching on Cognitive Performance and Related Cardiac Autonomic Control." *Scand J Med Sci Sports* 24 (1): 234–42.

Dupuy, O, Renaud, M, Bherer, L, and Bosquet, L. 2010. "Effect of Functional Overreaching on Executive Functions." *Int J Sports Med* 31 (9): 617–23.

Eijsvogels, TM., and Thompson, PD. 2017. "Are There Clinical Cardiac Complications from Too Much Exercise?" *Curr Sports Med Rep* 16 (1): 9–11.

Eijsvogels, TMH, Hoogerwerf, MD, Maessen, MFH, Seeger, JPH, George, KP, Hopman, MTE, and Thijssen, DHJ. 2015. "Predictors of Cardiac Troponin Release after a Marathon." *J Sci Med Sport* 18 (1): 88–92.

Eisenhofer, G, Rundquist, B, Äneman, A, Friberg, P, Dakak, N, Kopin, IJ, Jacobs, MC, and Lenders, JWM. 1995. "Regional Release and Removal of Catecholamines and Extraneuronal Metabolism to Metanephrines." *J Clin Endocrinol Metab* 80 (10): 3009–17.

Elenkov, IJ, Wilder, RL, Chrousos, GP, and Vizi, ES. 2000. "The Sympathetic Nerve—An Integrative Interface between Two Supersystems: The Brain and the Immune System." *Pharmacol Rev* 52 (4): 595–638.

Filaire, E, Legrand, B, Lac, G, and Pequignot JM. 2004. "Training of Elite Cyclists: Effects on Mood State and Selected Hormonal Responses." *J Sports Sci* 22 (11–12): 1025–33.

Foster, C. 1998. "Monitoring Training in Athletes with Reference to Overtraining Syndrome." *Med Sci Sports Exerc* 30 (7): 1164–68.

Foster, C, Hector, LL, Welsh, R, Schrager, M, Green, MA, and Snyder, AC. 1995. "Effects of Specific versus Cross-Training on Running Performance." *Eur J Appl Physiol* 70 (4): 367–72.

Freeman, ME, Kanyicska, B, Lerant, A, and Nagy, G. 2000. "Prolactin: Structure, Function, and Regulation of Secretion." *Physiological Reviews* 80 (4): 1523–1631.

Fries, E, Hesse, J, Hellhammer, J, and Hellhammer, DH. 2005. "A New View on Hypocortisolism." *Psychoneuroendocrinology* 30 (10): 1010–16.

Gastmann, UAL, and Lehmann MJ. 1998. "Overtraining and the BCAA Hypothesis." *Med Sci Sports Exerc* 30 (7): 1173–78.

Halson, SL. 2014. "Monitoring Training Load to Understand Fatigue in Athletes." *Sports Med* 44 (Suppl 2): S139-47.

Halson, SL, Bridge, MW, Meeusen, R, Busschaert, B, Gleeson, M, Jones, DA, and Jeukendrup, AE. 2002. "Time Course of Performance Changes and Fatigue Markers during Intensified Training in Trained Cyclists." *J Appl Physiol* 93 (3): 947–56.

Hasegawa, Y, Ljichi, T, Kurosawa, Y, Hamaoka, T, and Goto, K. 2015. "Planned Overreaching and Subsequent Short-Term Detraining Enhance Cycle Sprint Performance." *Int J Sports Med* 36: 666–71.

Hausswirth, C, Louis, J, Aubry, A, Bonnet, G, Duffield, R, and Le Meur, Y. 2014. "Evidence of Disturbed Sleep and Increased Illness in Overreached Endurance Athletes." *Med Sci Sports Exerc* 46 (5): 1036–45.

Hecksteden, A, Skorski, S, Schwindling, S, Hammes, D, Pfeiffer, M, Kellmann M, Ferrauti, A, and Meyer, T. 2016. "Blood-Borne Markers of Fatigue in Competitive Athletes - Results from Simulated Training Camps." *PLoS ONE* 11 (2): 1–13.

Hedelin, R, Kenttä, G, Wiklund, U, Bjerle, P, and Henriksson-Larsén, K. 2000. "Short-Term Overtraining: Effects on Performance, Circulatory Responses, and Heart Rate Variability." *Med Sci Sports Exerc* 32 (8): 1480–84.

Heim, C, Ehlert, U, and Hellhammer, DH. 2000. "The Potential Role of Hypocortisolism in the Pathophysiology of Stress-Related Bodily Disorders." *Psychoneuroendocrinology* 25 (1): 1–35.

Hoogerwaard, EM, Schouten, Y, van der Kooi, AJ, Gorgels, JP, de Visser, M, and Sanders, GT. 2001. "Troponin T and Troponin I in Carriers of Duchenne and Becker Muscular Dystrophy with Cardiac Involvement." *Clin Chem* 47 (5): 962–63.

Hopkins, WG, Hawley, JA, and Burke, LM. 1999. "Design and Analysis of Research on Sport Performance Enhancement." *Med Sci Sports Exerc* 31 (3): 472–85.

Hopkins, WG, Marshall, SW, Batterham, AM, and Hanin, J. 2009. "Progressive Statistics for Studies in Sports Medicine and Exercise Science." *Med Sci Sports Exerc* 41 (1): 3–12.

Hynynen, E, Uusitalo, A, Konttinen, N, and Rusko, H. 2008. "Cardiac Autonomic Responses to Standing up and Cognitive Task in Overtrained Athletes." *Int J Sports Med* 29 (7): 552–58.

Jansen, JJ, Hilvering, B, van den Doel, A, Pickkers, P, Koenderman, L, Buydens, LMC, and van den Brink, OF. 2016. "FLOOD: FLow Cytometric Orthogonal Orientation for Diagnosis." *Chemometrics and Intelligent Laboratory Systems* 151: 126–35.

Jeukendrup, AE, Hesselink, MKC, Snyder, AC, Kuipers, H, and Keizer, HA. 1992. "Physiological Changes in Male Competitive Cyclists after Two Weeks of Intensified Training." *Int J Sports Med* 13 (7): 534–41.

Jones, NL, Makrides, L, Hitchcock, C, Chypchar, T, and McCartney, N. 1985. "Normal Standards for an Incremental Progressive Cycle Ergometer Test." *Am Rev Respir Dis* 131 (5): 700–708.

Joyner, MJ, and Coyle, EF. 2008. "Endurance Exercise Performance: The Physiology of Champions." *J Physiol* 586 (1): 35–44.

Kamp, VM, Leentjens, J, Pillay, J, Langereis, JD, de Kleijn, S, Kox, M, Netea, MG, Pickkers, P, and Koenderman, L. 2013. "Modulation of Granulocyte Kinetics by GM-CSF/IFN-γ in a Human LPS Rechallenge Model." *J Leukoc Biol* 94 (3): 513–20.

Kellmann, M, Bertollom, M, Bosquet, L, Brink, M, Coutts, AJ, Duffield, R,

Erlacher, D, et al. 2018. "Recovery and Performance in Sport: Consensus Statement." *Int J Sports Physiol Perform* 13 (2): 240-245.

Kellmann, M, and Kallus KW. 2001. *The Recovery-Stress Questionnaire for Athletes: User Manual*. Champaign (IL): Human Kinetics.

Kenttä, G, Hassmén, P, and Raglin, JS. 2006. "Mood State Monitoring of Training and Recovery in Elite Kayakers." *Eur J Sports Sci* 6 (4): 245–53.

Kuipers, H, and Keizer, HA. 1988. "Overtraining in Elite Athletes. Review and Directions for the Future." *Sports Med* 6 (2): 79–92.

Lamberts, RP, Rietjens, GJ, Tijdink, HH, Noakes, TD, and Lambert, MI. 2010. "Measuring Submaximal Performance Parameters to Monitor Fatigue and Predict Cycling Performance: A Case Study of a World-Class Cyclo-Cross Cyclist." *Eur J Appl Physiol* 108 (1): 183–90

Lamberts, RP, Swart, J, Noakes, TD, and Lambert, MI. 2011. "A Novel Submaximal Cycle Test to Monitor Fatigue and Predict Cycling Performance." *Br J Sports Med* 45 (10): 797–804.

Lambourne, K, and Tomporowski, P. 2010. "The Effect of Exercise-Induced Arousal on Cognitive Task Performance: A Meta-Regression Analysis." *Brain Res* 1341: 12–24.

Le Meur, Y, Buchheit, M, Aubry, A, Coutts, AJ, and Hausswirth, C. 2017. "Assessing Overreaching with HRR: What Is the Minimal Exercise Intensity Required?" *Int J Sports Physiol Perform* 12 (4): 569-573.

Le Meur, Y, Hausswirth, C, Natta, F, Couturier, A, Bignet, F, and Vidal, PP. 2013. "A Multidisciplinary Approach to Overreaching Detection in Endurance Trained Athletes." *J Appl Physiol* 114 (3): 411–20.

Le Meur, Y, Louis, J, Aubry, A, Gueneron, J, Pichon, A, Schaal, K, Corcuff, JB, Hatem, SN, Isnard, R, and Hausswirth, C. 2014. "Maximal Exercise

Limitation in Functionally Overreached Triathletes: Role of Cardiac Adrenergic Stimulation." *J Appl Physiol* 117 (3): 214–22.

Le Meur, Y, Pichon, A, Schaal, K, Schmitt, L, Louis, J, Gueneron, J, Vidal, PP, and Hausswirth, C. 2013. "Evidence of Parasympathetic Hyperactivity in Functionally Overreached Athletes." *Med Sci Sports Exerc* 45 (11): 2061–71.

Lehmann, M, Dickhuth, HH, Gendrisch, G, Lazar, W, Thum, M, Kaminski, R, Aramendi, J, Peterke, E, Wieland, W, and Keul, J. 1991. "Training - Overtraining. A Prospective, Experimental Study with Experienced Middle- and Long-Distance Runners." *Int J Sports Med* 12 (5): 444–52.

Lehmann, M, Foster, C, Dickhuth, HH, and Gastmann, U. 1998. "Autonomic Imbalance Hypothesis and Overtraining Syndrome." *Med Sci Sports Exerc* 30 (7): 1140–45.

Lehmann, M, Gastmann, U, Petersen, KG, Bachl, N, Seidel, A, Khalaf, AN, Fischer, S, and Keul, J. 1992. "Training-Overtraining: Performance, and Hormone Levels, after a Defined Increase in Training Volume versus Intensity in Experienced Middle- and Long-Distance Runners." *Br J Sports Med* 26 (4): 234–42.

Lehmann, M, Knizia, K, Gastmann, U, Petersen, KG, Khalaf, AN, Bauer S, Kerp, L, and Keul, J. 1993. "Influence of 6-Week, 6 Days per Week, Training on Pituitary Function in Recreational Athletes." *Br J Sports Med* 27 (3): 186–92.

Lucía, A, Hoyos, J, Santalla, A, Earnest, C, and Chicharro, JL. 2003. "Tour de France versus Vuelta a España: Which Is Harder?" *Med Sci Sports Exerc* 35 (5): 872–78.

MacKinnon, LT. 2000. "Overtraining Effects on Immunity and Performance

in Athletes." *Immunol Cell Biol* 78 (5): 502–9.

Meeusen, R, Duclos, M, Foster, C, Fry, A, Gleeson, M, Nieman, D, Raglin, J, Rietjens, G, Steinacker, J, and Urhausen, A. 2013. "Prevention, Diagnosis, and Treatment of the Overtraining Syndrome: Joint Consensus Statement of the European College of Sport Science and the American College of Sports Medicine." *Med Sci Sports Exerc* 45 (1): 186–205.

Meeusen, R, Duclos, M, Gleeson, M, Rietjens, G, Steinacker, J, and Urhausen, A. 2006. "Prevention, Diagnosis and Treatment of the Overtraining Syndrome." *Eur J Sport Sci* 6 (1): 1–14.

Meeusen, R, Nederhof, E, Buyse, L, Roelands, B, de Schutter, G, and Piacentini MF. 2010. "Diagnosing Overtraining in Athletes Using the Two-Bout Exercise Protocol." *Br J Sports Med* 44 (9): 642–48.

Meeusen, R, Piacentini, MF, Busschaert, B, Buyse, L, De Schutter, G, and Stray-Gundersen, J. 2004. "Hormonal Responses in Athletes: The Use of a Two Bout Exercise Protocol to Detect Subtle Differences in (over) Training Status." *Eur J Appl Physiol* 91 (2–3): 140–46.

Micklewright, D, Papadopoulou, E, Parry, D, Hew-Butler, T, Tam, N, and Noakes, T. 2009. "Perceived Exertion Influences Pacing among Ultramarathon Runners but Post-Race Mood Change Is Associated with Performance Expectancy." *South Afr J Sports Med* 21 (4): 167–72.

Miller, J. 1982. "Discrete versus Continuous Stage Models of Human Information Processing: In Search of Partial Output." *J Exp Psychol Hum Percept Perform* 8 (2): 273–96.

Møller, N, and Jørgensen, JOL. 2009. "Effects of Growth Hormone on Glucose, Lipid, and Protein Metabolism in Human Subjects." *Endocr*

Rev 30 (2): 152–77.

Mujika, I, and Padilla, S. 2000. "Detraining: Loss of Training-Induced Physiological and Performance Adaptations. Part I." *Sport Med* 30 (2): 79–87.

Nässi, A, Ferrauti, A, Meyer, T, Pfeiffer, M, and Kellmann, M. 2017. "Development of Two Short Measures for Recovery and Stress in Sport." *Eur J Sport Sci* 17 (7): 894–903.

Nederhof, E, Lemmink, KAPM, Visscher, C, Meeusen, R, and Mulder. T. 2006. "Psychomotor Speed - Possibly a New Marker for Overtraining Syndrome." *Sports Med* 36 (10): 817–28.

Nederhof, E, Lemmink, KAPM, Zwerver, J, and Mulder. T. 2007. "The Effect of High Load Training on Psychomotor Speed." *Int J Sports Med* 28 (7): 595–601.

Nederhof, E, Zwerver, J, Brink, M, Meeusen, R, and Lemmink, K. 2008. "Different Diagnostic Tools in Nonfunctional Overreaching." *Int J Sports Med* 29 (7): 590–97.

Petibois, CG, Cazorla, G, Poortmans, JR, and Deleris, G. 2002. "Biochemical Aspects of Overtraining in Endurance Sports: A Review." *Sports Med* 32 (13): 867–78.

Piacentini, MF, and Meeusen, R. 2015. "An Online Training-Monitoring System to Prevent Nonfunctional Overreaching." *Int J Sports Physiol Perform* 10 (4): 524–27.

Piacentini, MF, Witard, OC, Tonoli, C, Jackman, SR, Turner, JE, Kies, AK, Jeukendrup, AE, Tipton, KD, and Meeusen, R. 2016. "Intensive Training Affects Mood with No Effect on Brain-Derived Neurotrophic

Factor." *Int J Sports Physiol Perform* 11 (6): 824–30.

Rietjens, GJWM, Kuipers, H, Adam, JJ, Saris, WHM, van Breda, E, Van Hamont, D, and Keizer, HA. 2005. "Physiological, Biochemical and Psychological Markers of Strenuous Training-Induced Fatigue." *Int J Sports Med* 26 (1): 16–26.

Robson, PJ. 2003. "Elucidating the Unexplained Underperformance Syndrome in Endurance Athletes : The Interleukin-6 Hypothesis." *Sports Med* 33 (10): 771–81.

Robson, PJ, Blannin, AK, Walsh, NP, Castell, LM, and Gleeson, M. 1999. "Effects of Exercise Intensity, Duration and Recovery on in Vitro Neutrophil Function in Male Athletes." *Int J Sports Med* 20 (2): 128–35.

Rodríguez-Marroyo, JA, García-López, J, Juneau, CE, and Villa, JG. 2009. "Workload Demands in Professional Multi-Stage Cycling Races of Varying Duration." *Br J Sports Med* 43 (3): 180–85.

Rodríguez-Marroyo, JA, Villa, JG, Pernía, R, and Foster, C. 2017. "Decrement in Professional Cyclists' Performance after a Grand Tour." *Int J Sports Physiol Perform* 12 (10): 1348–55.

Sanders, D, Heijboer, M, Hesselink, MKC, Myers, T, and Akubat, I. 2017. "Analysing a Cycling Grand Tour: Can We Monitor Fatigue with Intensity or Load Ratios?" *J Sports Sci* 36 (12): 1385-1391.

Satz, P. 1993. "Brain Reserve Capacity on Symptom Onset after Brain Injury: A Formulation and Review of Evidence for Threshold Theory." *Neuropsychology* 7 (3): 273–95.

Saw, AE, Kellmann M, Main, LC, and Gastin, PB. 2017. "Athlete Self-Report Measures in Research and Practice: Considerations for the Discerning

Reader and Fastidious Practitioner." *Int J Sports Physiol Perform* 12 (Suppl 2): 127–35.

Schmikli, SL, de Vries, WR, Brink, MS, and Backx, FJG. 2012. "Monitoring Performance, Pituitary-Adrenal Hormones and Mood Profiles: How to Diagnose Non-Functional over-Reaching in Male Elite Junior Soccer Players." *Br J Sports Med* 46 (14): 1019–23.

Smith, LL. 2000. "Cytokine Hypothesis of Overtraining: A Physiological Adaptation to Excessive Stress?" *Med Sci Sports Exerc* 32 (2): 317–31.

Smith, LL. 2003. "Overtraining, Excessive Exercise, and Altered Immunity: Is This a T Helper-1 versus T Helper-2 Lymphocyte Response?" *Sports Med* 33 (5): 347–64.

Snow, J. 1855. *On the Mode of Communication of Cholera*. 2nd editio. London: J. Churchill.

Snyder, AC. 1998. "Overtraining and Glycogen Depletion Hypothesis." *Med Sci Sports Exerc* 30 (7): 1146–50.

Steinacker, JM, Lormes, W, Kellmann, M, Liu, Y, Reißnecker. S, Opitz-Gress, A, Baller, B, et al. 2000. "Training of Junior Rowers before World Championships. Effects on Performance, Mood State and Selected Hormonal and Metabolic Responses." *J Sports Med Phys Fitness* 40 (4): 327–35.

Steinacker, JM., Lormes, W, Reissnecker, S, and Liu, Y. 2004. "New Aspects of the Hormone and Cytokine Response to Training." *Eur J Appl Physiol* 91 (4): 382–91.

Svendsen, IS., Killer, SC, Carter, JM, Randell, RK, Jeukendrup, AE, and Gleeson, M. 2016. "Impact of Intensified Training and Carbohydrate

Supplementation on Immunity and Markers of Overreaching in Highly Trained Cyclists." *Eur J Appl Physiol* 116 (5): 867–77.

Taylor, KL, Chapman, DW, Cronin, JB, Newton, MJ, and Gill, N. 2012. "Fatigue Monitoring in High Performance Sport: A Survey of Current Trends." *J Aust Strength Cond* 20 (1): 12–23.

ten Haaf, T, van Staveren, S, Oudenhoven, E, Piacentini, MF, Meeusen, R, Roelands, B, Koenderman, L, Daanen, HAM, Foster, C, and de Koning, JJ. 2017. "Prediction of Functional Overreaching from Subjective Fatigue and Readiness to Train after Only 3 Days of Cycling." *Int J Sports Physiol Perform* 12 (S2): 87–94.

Tsigos, C, and Chrousos, GP. 2002. "Hypothalamic-Pituitary-Adrenal Axis, Neuroendocrine Factors and Stress." *J Psychosom Res* 53 (4): 865–71.

Uhart, M, Chong, RY, Oswald, L, Lin, PI, and Wand, GS. 2006. "Gender Differences in Hypothalamic-Pituitary-Adrenal (HPA) Axis Reactivity." *Psychoneuroendocrinology* 31 (5): 642–52.

Urhausen, A, Gabriel, HHW, Weiler, B, and Kindermann, W. 1998. "Ergometric and Psychological Findings during Overtraining: A Long-Term Follow-up Study in Endurance Athletes." *Int J Sports Med* 19 (2): 114–20.

Urhausen, A, Gabriel, HH, and Kindermann, W. 1998. "Impaired Pituitary Hormonal Response to Exhaustive Exercise in Overtrained Endurance Athletes." *Med Sci Sports Exerc* 30 (3): 407–14.

Urhausen, A, and Kindermann, W. 2002. "Diagnosis of Overtraining: What Tools Do We Have?" *Sports Med* 32 (2): 95–102.

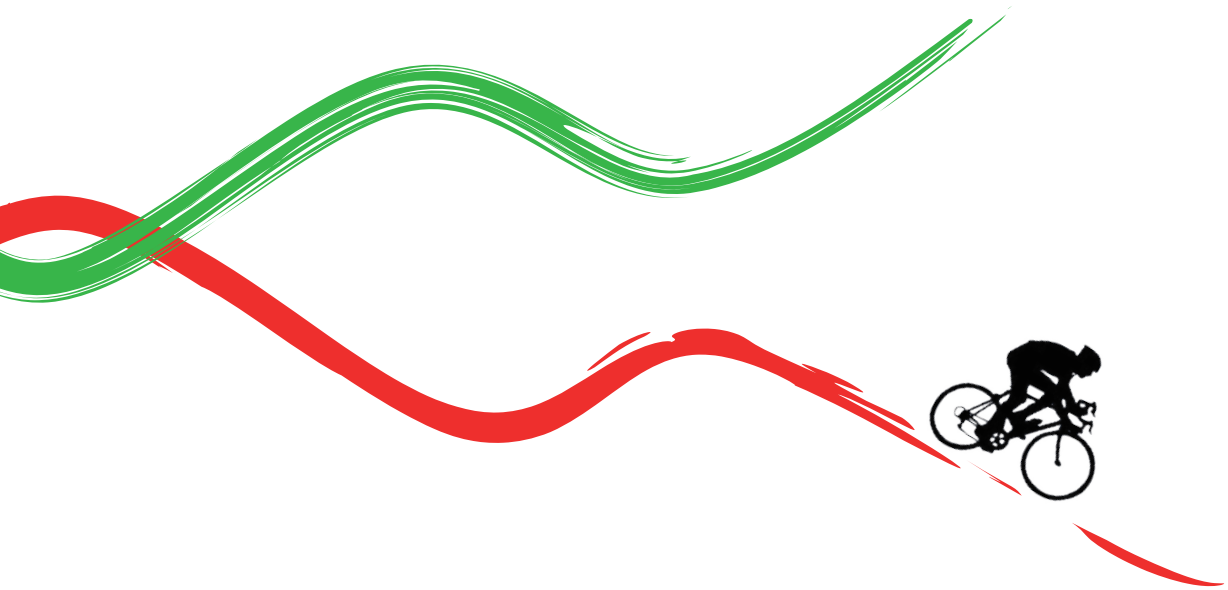
Uusitalo, ALT, Huttunen, P, Hanin, Y, Uusitalo, AJ, and Rusko, HK. 1998. "Hormonal Responses to Endurance Training and Overtraining in

Female Athletes." *Clin J Sport Med* 8 (3): 178–86.

Vrijkotte, S, Meeusen, R, Roelands, B, Kubesch, S, Mairesse, O, de Schutter, G, and Pattyn, N. 2017. "Refining Selection for Elite Troops by Predicting Military Training Outcome." *Aerosp Med Hum Perform* 88 (9): 850–57.

Wald, FDM, and Mellenbergh, GJ. 1990. "De Verkorte Versie van de Nederlandse Vertaling van de Profile of Mood States (POMS)." *Nederlands Tijdschrift Voor de Psychologie* 45: 86–90.

Summary



Periods of intensified training exert physiological and psychological stress on an athlete. If this so-called internal training load is balanced with sufficient recovery a period of intensified training results in acute fatigue (AF), characterized by increased performance capacity. Yet, increased internal training load that is not met by sufficient recovery results in a smaller increase or even reduced performance capacity. This process is termed overtraining and results in the possible outcomes functional overreaching (FOR), non-functional overreaching (NFOR), or overtraining syndrome (OTS). Besides a sub optimal effect of training (reduced or absent increase in performance), FOR has been associated with reduced sleep quality and increased risk for small illnesses. Moreover, a long-lasting stress-recovery imbalance may result in the more severe NFOR or eventually OTS, which need months to recover. Therefore, it is important to identify parameters that relate to underperformance (FOR) after intensified training. In this thesis we focused on some of the most promising parameters, namely hormones, heart rate, reaction time and monitoring of stressors and symptoms of overtraining. The Tour for Life (TFL), an 8-day non-competitive amateur cycling event that was the ecological source of this thesis, was applied as an experimental model. This TFL model was used to investigate whether hormonal levels, heart rate, reaction time or monitoring of stressors and symptoms can be used to distinguish between acute fatigue and functional overreaching. We defined the AF group as the group with no performance decrement and the FOR group as the group that underperformed after the TFL.

In chapter 2 it was investigated whether ACTH, cortisol, growth hormone, prolactin, metanephrine and normetanephrine levels were altered during the TFL. It was hypothesized that hormonal levels would change in FOR athletes, but not or to lesser extent in AF athletes. Blood samples were drawn in the afternoon immediately after the stage and the following morning at the start, halfway and end of the TFL. Overnight urine samples were collected the same mornings. None of the hormonal levels at the start, halfway and the end of the TFL were different between AF and FOR (all $P>.20$). On total group level

(AF+FOR), afternoon cortisol (P<.01) and growth hormone (P<.001) were increased from the start of the TFL. Morning ACTH, cortisol and prolactin decreased, whereas GH increased during the TFL (all P<.001). Metanephrine and normetanephrine levels did not change significantly (both P=.08). The change in growth hormone levels from the start to the end of the TFL were most strongly associated with the change in performance (afternoon: *r*=-.68, morning: *r*=-.63). This might point towards a relation between energy balance and change in performance.

Because it has been suggested that stimulation tests are more suitable than resting levels to demonstrate hormonal disturbances in the overtraining spectrum, a laboratory Two Bout Exercise Protocol has been applied in chapter 3. It was hypothesized that in FOR athletes 1) resting levels of ACTH, cortisol, GH and PRL would remain unchanged 1 and 5 weeks after the TFL, 2) exercise-induced hormonal responses would be reduced, most pronounced after the second exercise bout on a day, and 3) these changes would be absent or less noticeable in AF athletes. Two weeks before (pre TFL), 1 week after (post TFL) and 5 weeks after (follow-up) the TFL, subjects performed a maximal incremental cycling test in the morning and afternoon. Blood was drawn before and after each test and analysed for ACTH, cortisol, growth hormone and prolactin. It was shown that resting ACTH (P<.01) and GH (P<.001) were higher pre than post TFL. The response to the morning test was higher pre than post TFL for ACTH (P=.02), cortisol (P<.001) and PRL (P<.01), but not for GH (P=.12). The response to the afternoon test was only for cortisol higher pre than post TFL (P<.001). The results of the studies presented in chapter 2 and 3 clearly demonstrated a down-regulation of the hormonal system already in the early stages of the training-overtraining spectrum. Yet, the changes in the hormonal system were not different between AF and FOR athletes. This suggests that a down-regulation of the hormonal system is not the (single) performance limiting factor in FOR athletes.

Heart rate is often used to prescribe and monitor intensified training. However, it is unknown whether the change in heart rate is associated with the result of

intensified training, i.e. with the change in physical performance. In chapter 4 we evaluated cardiopulmonary exercise tests before and after the TFL. It was hypothesized that (sub) maximal heart rate would be lower after the TFL, but that this decrease would not be associated with a change in performance. In line with our hypothesis, post TFL heart rate was significantly reduced at low (-4.4 beats·min^{-1}, 95% CI [-8.7, -0.1]) and medium (-5.5 beats·min^{-1} [-8.5, -2.4]), but not at high intensity. Also peak heart rate was 3.4 beats·min^{-1} [-6.1, -0.7] lower post compared to pre TFL. In contrast, no changes in $\dot{V}O_2$ (P=.44) or the ventilator threshold (P=.21) were observed. Possibly, the decreased heart rate was compensated by an increased stroke volume or arteriovenous oxygen difference, as indicated by an increased O_2pulse (0.49 ml O_2·beat^{-1} [0.09, 0.89]). No differences between FOR and AF were observed for heart rate (P=.51). These results of this chapter suggest that heart rate is inadequate to prescribe and monitoring intensified training.

In chapter 5 we investigated whether choice reaction time can be used as a monitoring tool to establish overreaching. Reaction time has been suggested to be a practical monitoring tool that can easily be implemented in sports practice. Yet, until now, the limited available data is ambiguous. Our results showed that reaction time at the end of the TFL was 68 ms (95% CI [46, 89]) faster than at the start. During the laboratory assessments, reaction time post TFL (41 ms, 95% CI [12, 71]) and at follow-up (55 ms, 95% CI [26, 83]) were faster than pre TFL. The time by class interaction was not significant during (P=.26) and after (P=.43) the TFL. Also, correlations between physical performance and reaction time were not significant (all P>.30). It was, therefore, concluded that choice reaction time is likely not a useful tool to distinguish between AF and FOR.

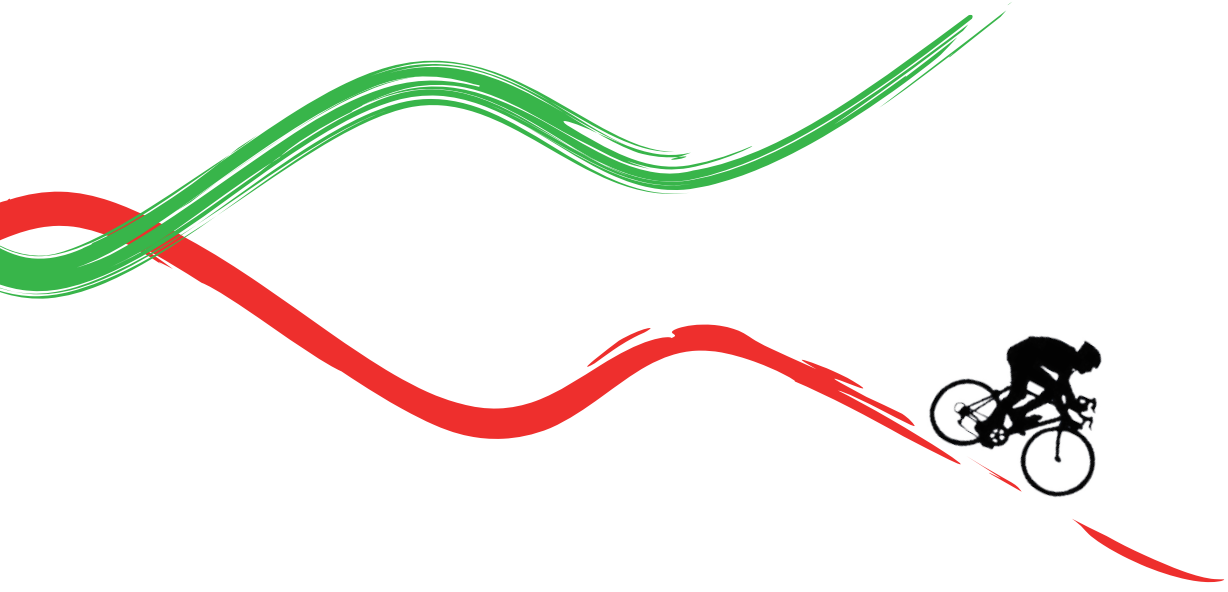
In chapter 6 we combined the Rating of Perceived Exertion, items from a validated online training monitor system, the Profile of Mood States questionnaire (POMS), resting heart rate and rectal temperature to study which (combination of) parameters can be used to early distinguish between AF and FOR. These easily measurable stressors and symptoms were

monitored during the TFL. The combination of subjective rating of fatigue and readiness to train on visual analogue scales (VAS) were most powerful to distinguish between AF and FOR. After three days cycling these 2 parameters correctly predicted 78% of the subjects as AF or FOR (sensitivity=79%, specificity=77%).

In chapter 7 it was concluded that we did not identify a single performance-limiting factor in amateur cyclists after an 8-day non-competitive event. Instead, monitoring of the stress-recovery balance (which reflects the underlying process of the overtraining spectrum) by means of subjective ratings of fatigue and readiness to train was shown most powerful to distinguish between AF and FOR. Practitioners are, therefore, encouraged to incorporate frequent monitoring of the stress-recovery balance in supervision of their athletes. They are advised to adapt their methodology so that it best fits the situation and desires of the athletes and coaches.

Dutch summary

(Nederlandse samenvatting)



Periodes van intensieve inspanning veroorzaken fysieke en psychologische stress op een atleet. Als deze zogenoemde interne trainingsbelasting in verhouding is met voldoende herstel, dan resulteert deze periode van intensieve training in acute vermoeidheid (AV), gekenmerkt door een verhoogde inspanningscapaciteit. Echter, een periode van intensieve training zonder voldoende herstel resulteert in minder of zelfs helemaal geen prestatieverbetering. Dit proces noemt men overtraining, wat resulteert in de mogelijke uitkomsten functionele overreaching (FOR), niet-functionele overreaching (NFOR) of overtrainingssyndroom (OTS). Naast een suboptimaal effect van training (beperkte of volledig afwezige toename van prestatie) wordt FOR geassocieerd met verminderde slaapkwaliteit en een verhoogd risico op ziekten. Bovendien resulteert een langdurige onbalans tussen stress en herstel in NFOR en uiteindelijk in OTS, waarvan het herstel maanden kan duren. Het is daarom van belang om parameters te identificeren die gerelateerd zijn aan een afname van prestatie na intensieve training (FOR). In dit proefschrift werd gefocust op enkele van de meest belovende parameters, namelijk hormonen, hartfrequentie, reactietijd en het monitoren van stressoren en symptomen van overtraining. De Tour for Life (TFL), een 8-daags niet-competitief evenement voor amateur wielrenners en de inspiratiebron van dit proefschrift, werd gebruikt als een experimenteel model. Dit TFL model werd gebruikt om te onderzoeken of hormonen, hartfrequentie, reactietijd of het monitoren van stressoren en symptomen van overtraining gebruikt kunnen worden om onderscheidt te maken tussen acute vermoeidheid en functionele overreaching. De acute vermoeidheid groep (AV) werd gedefinieerd als de groep deelnemers zonder afname in inspanningscapaciteit en de FOR groep als de deelnemers met verminderde prestatie na de TFL.

In hoofdstuk 2 werd onderzocht of ACTH, cortisol, groeihormoon, prolactine, metanefrine en normetanefrine concentraties veranderen tijdens de TFL. De hypothese was dat deze hormonale concentraties zouden veranderen in de FOR groep maar niet, of in mindere mate, in de AV groep. Aan het begin, halverwege en einde van de TFL werd direct na de finish in de namiddag en

de daaropvolgende ochtend bloed afgenomen. Daarnaast werd op dezelfde tijdspunten de nachtelijke urine verzameld. Gemiddeld over alle deelnemers (AV + FOR groep) werd een stijging van cortisol (P<.01) en groeihormoon (P<.001) ten opzichte van de start van de TFL waargenomen. Daarnaast waren de ACTH, cortisol en prolactine concentratie in de ochtend lager, en de groeihormoon concentratie hoger ten opzichte van de start (alle P<.001). Metanefrine en normetanefrine concentraties waren onveranderd (P=.08). Echter, geen van de hormonale waarden aan de start, halverwege of einde van de TFL was verschillend tussen de AV en FOR groep (alle P>.20). De verandering in groeihormoon tussen de start en het einde van de TFL was het sterkst geassocieerd met de verandering in prestatie (namiddag: r=-.68, ochtend: r=-.63). Dit wijst mogelijk op een relatie tussen de energiebalans en de verandering in prestatie.

Omdat in de literatuur gesuggereerd is dat stimulatie testen geschikter zijn dan rustwaarden om hormonale veranderingen in het overtraining spectrum aan te tonen, werd in hoofdstuk 3 een zogenoemd Dubbel Inspanningsprotocol toegepast. De hypothesen waren dat (1) de FOR groep op 1 en 5 weken na de TFL onveranderde rustwaarden voor ACTH, cortisol, groeihormoon en prolactine zouden hebben, (2) een verminderde hormonale response op inspanning zou hebben, met name tijdens de tweede inspanningstest op een dag, en (3) deze veranderingen zouden niet of in mindere mate aanwezig zijn in de AV groep. De deelnemers voerden 2 weken voor (pre TFL), 1 week na (post TFL) en 5 weken na (follow-up) de TFL een maximale inspanningstest uit in de ochtend en de middag. Bloed werd afgenomen voor en na iedere inspanningstest en hierin werden ACTH, cortisol, groeihormoon en prolactine waarden gemeten. De rustwaarden van ACTH (P<.01) en groeihormoon (P<.001) waren hoger pre dan post TFL. De hormonale response op de ochtend inspanningstest pre TFL was groter dan post TFL voor ACTH (P=.02), cortisol (P<.001) en prolactine (P<.01), maar onveranderd voor groeihormoon (P=.12). De response op de middag inspanningstest was alleen voor cortisol hoger pre TFL dan post TFL (P<.001). De resultaten

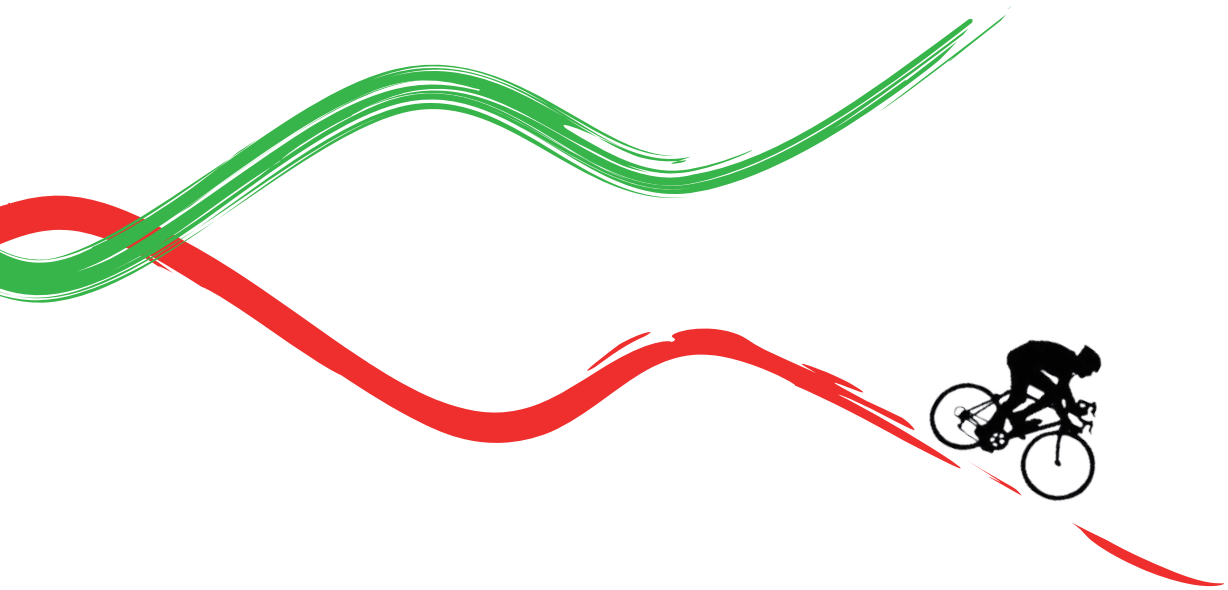
van hoofdstuk 2 en 3 tonen duidelijk aan dat al in het vroege stadium van het overtraining spectrum (AV en FOR) het hormonale systeem onderdrukt wordt. Echter, geen van de waargenomen veranderingen was verschillend tussen de AV en de FOR groep. Dit suggereert dat veranderingen in het hormonale systeem niet de prestatiebelemmerende factor is in FOR atleten.
Hartfrequentie wordt vaak gebruikt om intensieve training voor te schrijven en te monitoren. Echter, het is onbekend of de verandering in hartfrequentie geassocieerd is met het resultaat van intensieve training, dat wil zeggen, met de verandering in inspanningscapaciteit. In hoofdstuk 4 hebben we dit onderzocht met behulp van cardiopulmonaire inspanningstesten voor en na de TFL. De hypothese was dat sub (maximale) hartfrequentie lager zou zijn na de TFL, maar dat deze verandering niet geassocieerd was met de verandering in prestatie. In lijn met onze hypothese werd aangetoond dat de hartfrequentie post TFL lager was op lage (-4.4 slagen·min-1, 95% CI [-8.7, -0.1]) en matige (-5.5 slagen·min-1 [-8.5, -2.4]) intensiteit, maar niet op hoge intensiteit. De piek hartfrequentie was 3.4 slagen·min-1 [-6.1, -0.7] lager post dan pre TFL. Daarentegen waren de zuurstofopname (P=.44) en ventilatoire drempel (P=.21) onveranderd. Mogelijk werd de lagere hartfrequentie gecompenseerd door een hoger slagvolume of arterioveneus zuurstof verschil, zoals gesuggereerd door een hogere O2pulse (0.49 ml O2·slag-1 [0.09, 0.89]). Tot slot werden geen verschillen in hartfrequentie gevonden tussen de AV en FOR groep (P=.51). De resultaten van dit hoofdstuk suggereren dat hartfrequentie ongeschikt is om training voor te schrijven en te monitoren tijdens perioden van intensieve training.
In hoofdstuk 5 hebben we onderzocht of reactietijd kan worden gebruikt als een instrument om overreaching te detecteren. In de literatuur is gesuggereerd dat reactietijd een praktisch toepasbaar monitoring instrument kan zijn dat makkelijk geïmplementeerd kan worden in de sportpraktijk. Echter, tot nu toe is weinig data gepubliceerd, en de gerapporteerde resultaten zijn niet consistent. Onze resultaten laten zien dat reactietijd aan het eind van de TFL 68 ms (95% CI [46, 89]) sneller was dan aan de start. De metingen in

het laboratorium toonden aan dat de reactietijd post TFL (41 ms, 95% CI [12, 71]) en tijdens de follow-up meting (55 ms, 95% CI [26, 83]) sneller was pre TFL. De interactie tussen tijd en groep was niet significant tijdens (P=.26) en na (P=.43) de TFL. Bovendien waren de correlaties tussen de verandering in prestatie en reactietijd niet significant (alle P>.30). Daarom werd geconcludeerd dat reactietijd waarschijnlijk geen bruikbaar instrument is om onderscheidt te maken tussen AV en FOR atleten.

In hoofdstuk 6 combineerden we Rating of Perceived Exertion (score van ervaren vermoeidheid), items van een gevalideerd online training monitor systeem, de Profile of Mood States vragenlijst (POMS), hartfrequentie in rust, en rectaal temperatuur om te onderzoeken welke (combinatie van) parameters het meest geschikt was op in vroegtijdig stadium onderscheidt te kunnen maken tussen AV en FOR atleten. Deze eenvoudig te meten stressoren en symptomen van overtraining werden gemonitord tijdens de TFL. De combinatie van subjectief beoordeelde vermoeidheid en gereedheid om te trainen, gescoord op een visuele analoge schaal (VAS), resulteerde in het beste onderscheidt tussen de AV en FOR groep. Met behulp van de combinatie van deze 2 parameters kon 78% van de deelnemers na 3 fietsdagen correct geclassificeerd worden als AV of FOR atleet (sensitiviteit=79%, specificiteit=77%).

In hoofdstuk 7 werd geconcludeerd dat we niet 1 specifieke prestatie belemmerende factor geïdentificeerd hebben in amateurwielrenners na een 8-daags niet-competitief evenement. In plaats daarvan is gebleken dat het monitoren van de stress-herstel balans (die ten grondslag ligt aan overtraining) door middel van subjectief beoordeelde vermoeidheid en gereedheid om te trainen het meest geschikt was om onderscheidt te maken tussen AV en FOR atleten. Daarom worden begeleiders van atleten aangemoedigd om regelmatige monitoring van de stress-herstel balans te integreren in hun ondersteuning van hun sporters. Men wordt geadviseerd om hun methode aan te passen aan de situatie en de wensen van atleten en hun coaches.

List of publications



Ten Haaf, T & Weijs, P. 2014. "Resting Energy Expenditure Prediction in Recreational Athletes of 18–35 Years: Confirmation of Cunningham Equation and an Improved Weight-Based Alternative." *PlosOne*, doi: 10.1371/journal.pone.0108460.

De Koning, JJ, **ten Haaf, T**, van Staveren, S, Roelands, B, Foster, C, Piacentini, MF, & Meeusen, R. 2015. "An investigation of the underlying mechanisms of overtraining in a natural experimental model." *J Sci Cycling* 42(2): 36-37.

Ten Haaf, T, van Staveren, S, Oudenhoven, E, Piacentini, MF, Meeusen, R, Roelands, B, Koenderman, L, Daanen, HAM, Foster, C, & de Koning, JJ. 2017. "Prediction of Functional Overreaching From Subjective Fatigue and readiness to train after only 3 days of cycling." *Int J Sports Physiol Perform* 12(S2): 87-94.

Ten Haaf, T, van Staveren, S, Iannetta, D, Roelands, B, Meeusen, R, Piacentini, MF, Foster, C, Koenderman, L, Daanen, HAM, & de Koning, JJ. 2018. "Changes in choice reaction time during and after 8 days exhaustive cycling are not related to changes in physical performance." *Int J Sports Physiol Perform* 13(4): 428-33.

Ten Haaf, T., Verreijen, AM, Memelink, RG, Tieland, M, & Weijs, PJM. 2018. "Reduction in energy expenditure during weight loss is higher than predicted based on fat free mass and fat mass in older adults." *Clin Nutr* 37(1): 250-53.

Van Staveren, S, **ten Haaf, T**, Klöpping, M, Hilvering, B, Tinnevelt, GH, de Ruiter, K, Piacentini, MF, Roelands, B, Meeusen, R, de Koning, JJ, Jansen, JJ, Vrisekoop, N, & Koenderman, L. 2018. "Multi-dimensional flow cytometry analysis reveals increasing changes in the systemic

neutrophil compartment during seven consecutive days of endurance exercise." *Plos One*, doi: 10.1371/journal.pone.0206175.

Ten Haaf, T, van Staveren, S, Meeusen, R, Roelands, B, Piacentini, MF, Koenderman, L, Foster, C, & de Koning, JJ. "Hormonal changes in amateur athletes during an 8-day non-competitive cycling event." (*submitted for publication*)

Ten Haaf, T, Foster, C, Meeusen, R, Roelands, B, Piacentini, MF, van Staveren, S, Koenderman, L, & de Koning, JJ. "Heart rate seems inadequate to prescribe and monitor intensified training." (*submitted for publication*)

Ten Haaf, T, de Koning, JJ, van Staveren, S, Roelands, B, Piacentini, MF, Koenderman, L, Foster, C, & Meeusen, R. "Reduced exercise-induced ACTH, cortisol and prolactin responses after an 8-day non-competitive amateur cycling event." (*submitted for publication*)

In Dutch

Ten Haaf, T, Ijzerman, TH, & Savelberg, HHCM. 2011. "KinesioTaping en spierkracht; een sterk verhaal?" *Sport&Geneeskunde* 3:14-18.

Noordhof, DA, **ten Haaf, T**, Bolster, T, Kaagman, B, & Weijs, PJM. 2017. Juiste timing eiwitinname verkleint ziektekans sporters. *Sportgericht* 71(3): 14-18.

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...

About the author

Twan ten Haaf was born on March 20 1989 in Mill, the Netherlands. After finishing high school in 2007 he started studying General Health Sciences at Maastricht University. He specialized in Human Movement Sciences and Human Biology and graduated as a top 3% student of the university in 2010. After a gap-year he enrolled in the Research Master Human Movement Sciences at the Vrije Universiteit Amsterdam. He investigated the reliability of a method to estimate gross efficiency during anaerobic exercise, and graduated cum laude in 2013. He then started working as a junior researcher on a project on overtraining under supervision of dr. Jos de Koning at the Vrije Universiteit Amsterdam. This was followed by a PhD study on this topic, of which the results are described in this thesis. Meanwhile, he worked as a researcher on weight management in collaboration with dr.ir. Peter Weijs at the Amsterdam University of Applied Sciences between 2014 and 2016. He is currently working as a sports scientist within the Chinese Olympic Committee speed skating program.

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